CHRONIC TOXICITY SUMMARY

SELENIUM AND SELENIUM COMPOUNDS

Molecular Formula	*Synonyms*	*Molecular Weight*	*CAS Reg. No.*
Se	elemental selenium	78.96 g/mol	7782-49-2
SeO_2	selenium dioxide; selenium oxide; selenious anhydride	110.96 g/mol	7446-08-4
H_2SeO_3	selenious acid	128.97 g/mol	7783-00-8
H_2Se	hydrogen selenide; selenium hydride; selenium anhydride	80.98 g/mol	7783-07-5
$SeOCl_2$	seleninyl chloride; selenium oxychloride; selenium oxichloric	165.86 g/mol	7791-23-3
Na_2SeO_3	disodium selenite	263.01 g/mol	10102-18-8
Na_2SeO_4	disodium selenate	188.94 g/mol	13410-01-0
SeS	selenium sulfide; sulfur selenide	111.02 g/mol	7446-34-6

I. Chronic Toxicity Summary

Inhalation reference exposure level	**0.08 µg Se/m^3**
Critical effect(s)	Respiratory effects; increased mortality in guinea pigs
Hazard index target(s)	Respiratory system

II. Chemical Property Summary (HSDB, 1995; Weast, 1980; Canady and Hodes, 1994; ACGIH, 1992)

Molecular formula	see above
Molecular weight	see above
Description	Se^0 crystal: metallic gray H_2Se: colorless gas H_2SeO_4, Na_2SeO_3: white crystals H_2SeO_3, Na_2SeO_4: colorless crystals SeO_2: lustrous crystals; yellow vapor SeS: yellow to orange powder
Vapor pressure	Se^0: 0.001 mm Hg at 20°C; 1 mm Hg at 356°C H_2Se: 760 mm Hg at -41.1°C SeO_2: 1 mm Hg at 157°C

Solubility	Se^0 : insol. in water, alcohol; sl. sol. in CS_2; sol. in ether H_2Se: 270 ml/100 ml water at 22.5°C; sol. in CS_2, carbonyl chloride H_2SeO_4: sol. in water; decomposes in alcohol H_2SeO_3: sol. in hot water, alcohol Na_2SeO_3: sol. in water Na_2SeO_4: 84 g/100 ml water at 35°C SeO_2: 38.4 g/100 ml water at 14°C SeS: insol. in water
Conversion factor	Se^0: not applicable (particulate) H_2Se: 3.3 µg/m^3 per ppb at 25ºC SeO_2: 4.5 µg/m^3 per ppb at 20°C

III. Major Uses and Sources

Selenium occurs in four valence states: selenates (Se^{6+}), selenites (Se^{4+}), selenides (Se^{2-}), and elemental selenium (Se^0) (Goyer, 1991) which include compounds formed with oxygen, sulfur, metals, and/or halogens. Selenium compounds are used in the glass industry as decolorizing agents and in the rubber industry as vulcanizing agents. Selenium compounds are also found in toning baths used in photography and xerography, and in insecticides and photoelectric cells. Selenium sulfide is used in shampoos as an anti-dandruff agent. The most widely used selenium compound in industry is selenium dioxide (SeO_2) which catalyzes reactions of organic compounds and is produced by the oxidation of selenium with nitric acid followed by evaporation or by burning selenium in oxygen (HSDB, 1995). Hydrogen selenide is formed by the reaction of acids or water with metal selenides or the contact of nascent hydrogen with soluble selenium compounds (Gingell *et al.*, 1982). Hydrogen selenide itself does not have a reported commercial use. The largest anthropogenic sources of atmospheric selenium are from the combustion of fossil fuels and the production/refining of copper, with the particulates the primary expected form of the compound (National Academy of Sciences (NAS), 1976; U.S. EPA, 1984).

Selenium is an essential trace element in humans and other species, with deficiency leading to cardiomyopathy in humans (Goyer, 1991). For dietary intake, the National Research Council has set a US Recommended Daily Allowance (RDA) of 0.87 µg/kg (55-70 µg/person/day) (Subcommittee on the Tenth Edition of the RDAs, 1989). The average daily oral intake of selenium is 125 µg/person (U.S. EPA, 1991).

IV. Effects of Human Exposures

Humans occupationally exposed to H_2Se gas at concentrations of 0.21 ppm H_2Se (0.7 mg Se/m^3) showed signs of acute toxicity (Buchan, 1947). Symptoms included irritation of the respiratory

tract, severe bronchitis, bronchial pneumonia, and pulmonary edema. Exposure duration was not stated.

Acute occupational exposure to SeO_2 gas resulted in bronchospasms, irritation of the upper respiratory passages, violent coughing and gagging with nausea and vomiting (Wilson, 1962).

The relationship between inhalation exposure to selenium and the presence of selenium in the urine was investigated in a five year study of workers at a selenium rectifying plant (Glover, 1967). Workers were exposed to fumes and dusts of elemental red selenium which the author reported is converted 80% to SeO_2 in the presence of air. Average air concentrations of selenium were reported to be 3.6 mg/m^3 in grinding processes, 0.04 mg/m^3 in annealing processes, and a range of averages of 0.23-0.87 mg Se/m^3 in various "special", punching, scraping, sorting, refining and testing processes. The same author previously reported symptoms among selenium exposed workers including garlic-like odor of the breath, skin rashes, indigestion, and poorly-defined "socio-psychological" effects including lassitude and irritability (Glover, 1954).

Clinical signs of toxicity were observed among a population exposed to high levels of selenium in soils and food supplies in China (Yang *et al.*, 1983). Approximately half of 248 people in this region showed symptoms including hair and nail loss, discoloration and decay of the teeth, and CNS disturbances including pain and anesthesia of the extremities. Animals in the region were also affected, with hoof damage and horn sloughing reported in water buffalo, cattle, and pigs. Populations in low-, medium-, and high-selenium areas of China were later studied to associate the symptoms with selenium intake. Estimated daily intake for adults in these areas were 70, 195, and 1438 μg Se for males and 62, 198, and 1238 μg for females, respectively (Yang *et al.*, 1989). Selenium intake was highly correlated with whole blood, breast milk, and 24-hour urine selenium levels. The authors also suggested the possibility of liver dysfunction as indicated by a delay in prothrombin time among persons with intake of 750-850 μg Se/day. More clearly recognized and characteristic clinical signs, however, were only observed in a group exposed to greater than 1261 μg Se/day and not among those exposed to less than 853 μg Se/day. Assuming a 55 kg body weight, these respective daily dose rates were 0.023 and 0.015 mg/kg-day.

A total population of 142 subjects in seleniferous areas of South Dakota and Wyoming was examined for signs of selenosis over a two-year period with monitoring of selenium levels in whole blood, serum, urine, and toenails (Longnecker *et al.*, 1991). Average intake among the population was estimated at 239 μg Se/day. No clinical signs and no changes in hematological function, clinical chemistry, or liver function were observed in the population.

V. Effects of Animal Exposures

Toxic effects from inhalation exposure to selenium dust were examined in rats, guinea pigs, and rabbits (Hall *et al.*, 1951). Twenty female rats were exposed for 8 hours to 33 ± 10 mg Se/m^3. Many animals showed signs of pulmonary effects at both one and 4 weeks after exposure, however, no control group was included in the experiment with which to compare incidence.

Similarly, six female rabbits and 10 male guinea pigs exposed to the same level of selenium dust for four 4-hour periods every 48 hours (8 days total duration) showed signs of interstitial pneumonitis at one week (2 animals of each species) and lung congestion and alveolar infiltration of large macrophages.

Guinea pigs exposed to concentrations "less than 0.021 mg H_2Se/l" (22 mg Se/m^3 as hydrogen selenide) for 2, 4, or 8 hours exhibited difficulty breathing and a red-tinged discharge from the nose (Dudley and Miller, 1941). Mortality studies were conducted with guinea pigs (16/group) using the same exposure durations and selenium concentrations ranging from 1 to 43 mg Se/m^3. Fifty percent mortality was observed at 30 days among animals exposed to 12 mg Se/m^3. Mortality after 30 days was 50% among animals exposed to 1 mg Se/m^3 for 8 hours. Histopathological evaluation of guinea pigs exposed for 4 hours to 8 mg Se/m^3 showed fatty change to the liver, pneumonia, lymphoid hyperplasia, and increased reticuloendothelial tissue in the spleen. These effects did not begin to resolve until more than 17 days after the exposure.

The absorption of selenium following inhalation exposure was studied in Fischer rats (Medinsky *et al.*, 1981). Male and female rats were exposed for 10 minutes in nose-only exposure chambers to aerosols at 2.6 mg Se/m^3 of either H_2SeO_3 (Se^{4+}) or elemental (red) selenium containing radioactive ^{75}Se. The compounds were also painted on the pelt in an effort to examine the contribution of dermal absorption from selenium deposited by the aerosols during the course of inhalation exposure. In the case of elemental selenium administration, the largest fractions of the initial body burden distributed to the pelt (39%), large intestine (15%), small intestine (9.3%), liver (9.1%), and blood (7.9%), with only 5% remaining in the lung. In the case of H_2SeO_3, distribution was to the pelt (55%), liver (13%), blood (5.6%), small intestine (3.2%), and kidney (1.8%), with 1.4% of the original body burden remaining in the lung. The calculated total absorption of the administered dose 4 hours after exposure was 94% for H_2SeO_3 and 57% for elemental Se. Studies in dogs also demonstrated extensive absorption of selenium metal and selenious acid aerosols to the gastrointestinal tract, blood and nasal membranes following inhalation exposure (Weissman *et al.*, 1983).

Several studies have addressed the toxicity of selenium compounds to animals when administered in either food or drinking water. Mice (50/group) treated with 0, 1, 4, or 8 ppm Na_2SeO_3 in drinking water over 50 weeks showed decreased growth rates at 8 ppm (Jacobs and Forst, 1981). The same group reported gross liver pathology in male mice treated by oral gavage for 3 days with 0.5 ml of 64 ppm Na_2SeO_3. Hamsters (8/sex/group) treated with 0.1 (unsupplemented), 1, 5, 10, or 20 ppm Na_2SeO_3 in the diet for 42 days showed histopathological changes to the liver (Beems and van Beek, 1985). Rats (6-8/group) treated in the diet with SeS_2, Na_2Se, Na_2SeO_3, or Na_2SeO_4 showed increased relative liver weights and/or decreased body weight gain at 10 ppm (for each compound) over a 5 week exposure (Dausch and Fullerton, 1993). A 13-week drinking water study of Na_2SeO_3, and Na_2SeO_4 in rats and mice showed increased mortality, decreased body weights, and histopathological changes to the kidneys in rats and decreased body weight and decreased water consumption in mice (Abdo, 1994). Decreased body weights were observed in rats treated for 6 weeks in drinking water with 2 ppm Na_2SeO_3 or Na_2SeO_4 (Palmer and Olson, 1974).

Decreased percentage of live spermatozoa, altered sperm morphology, and decreased body weight gain were observed in rats (6/group) treated for 5 weeks with 2 ppm Na_2SeO_3 in the diet (Kaur and Parshad, 1994). Rats (7-12/group) exposed to 0, 4, 8, or 16 ppm Na_2SeO_3 in drinking water for 240 days showed alterations in testicular LDH and β-glucuronidase activity at 4 ppm (Nebbia *et al.*, 1987).

Developmental toxicity endpoints were examined in hamsters (5-10/group) exposed by oral gavage on gestational day 8 to Na_2SeO_3 and Na_2SeO_4 at concentrations ranging from 0-110 μmol/kg body weight (Ferm *et al.*, 1990). Effects observed at 100 μmol Na_2SeO_3/kg included decreased fetal crown-rump length and increased percentage of abnormal litters. At 90 μmol Na_2SeO_4/kg, an increased percentage of abnormal litters was observed. Mice (10 or 14/group) treated with 0, 3, or 6 ppm Na_2SeO_3 in drinking water from 30 days pre-gestation through gestation showed altered estrus cycle length, decreased fetal growth, and a decreased number of ossified vertebrae in offspring (Nobunaga *et al.*, 1979).

VI. Derivation of Chronic Reference Exposure Level (REL)

Derivation of Chronic Inhalation Reference Exposure Level (REL)

Study	Dudley and Miller, 1941
Study population	Guinea pigs
Exposure method	Inhalation exposure
Critical effects	Respiratory irritation; bronchopneumonia; increased mortality
LOAEL	1 mg/m^3 (Se as H_2Se)
NOAEL	Not observed
Exposure continuity	Continuous
Exposure duration	8 hours
Average experimental exposure	1 mg/m^3 for LOAEL group
Human equivalent concentration	0.23 mg/m^3 for LOAEL group (gas with tracheobronchial and pulmonary respiratory effects, RGDR = 0.23 based on BW = 435 g, MV = 0.20 L/min, SA(TB) = 200 cm^2)
Subchronic uncertainty factor	10
LOAEL uncertainty factor	10
Interspecies uncertainty factor	3
Intraspecies uncertainty factor	10
Cumulative uncertainty factor	3,000
Reference exposure level	0.00008 mg/m^3 (0.08 $\mu g/m^3$)

Strengths of the selenium REL include the use of measured inhalation exposure data. Weaknesses include the difficulty in estimating effects of particulate selenium from data on

hydrogen selenide, the very short-term exposure involved, the lack of a NOAEL, and the lack of human data.

There are insufficient data relating human inhalation exposure to selenium compounds to adverse health effects for the development of a chronic REL although toxicity has been reported from occupational exposure to gases of both H_2Se and SeO_2 (Buchan, 1947; Wilson, 1962). Experiments in animals have shown that H_2Se is toxic following inhalation exposure, with 8-hour exposures to concentrations as low as 1 mg H_2Se/m^3 causing "irritation sufficiently damaging to cause pneumonitis" and subsequently increasing 30-day mortality (Dudley, 1937; Dudley and Miller, 1941). The Dudley and Miller (1941) study was selected as the basis for the development of the chronic REL. Although of short duration, the toxicity of H_2Se is high enough relative to other selenium compounds that it is important to protect against possible adverse effects from exposure to this compound. There are no long-term studies on the toxicity of H_2Se.

VII. References

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CHRONIC TOXICITY SUMMARY

METALLIC SILVER AND SILVER COMPOUNDS

Molecular formula	*Molecular weight*	*Compound*	*CAS Registry Number*
Ag	107.87	silver, argentum	7440-22-4
$AgNO_3$	169.89	silver nitrate, nitric acid silver (I) salt	7761-88-8
Ag_2O	231.8	silver (I)oxide	20667-12-3
AgO	123.88	silver (II) oxide, silver peroxide	1301-96-8, 35366-11-1
Ag_2S	247.80	silver sulfide, acanthite	21548-73-2
AgCl	143.34	silver chloride, silver monochloride	7783-90-6
AgI	234.80	silver iodide	7783-96-2

I. Chronic Toxicity Summary

Inhalation reference exposure level	**20 μg Ag/m^3**
Critical effect(s)	Argyria in humans
Hazard index target(s)	Respiratory system; alimentary system; eyes

II. Physical and Chemical Properties (For metallic silver except as noted) (ATSDR, 1990)

Molecular formula	Ag
Molecular weight	107.87
Description	Ag metal: Lustrous, white solid metal $AgNO_3$: Colorless/white crystalline solid Ag_2S: Gray-black solid AgCl: White solid Ag_2O: Dk. brown to black crystalline solid AgO: Charcoal gray powder, black crystal
Specific gravity	10.5
Boiling point	2212°C @ 760 mmHg
Vapor pressure	100 mm Hg @ 1,865°C
Solubility	Ag metal: Soluble in nitric acid & potassium cyanide $AgNO_3$: Soluble in water, ethanol AgCl: Insoluble in water Ag_2O: Slightly soluble in water, alcohol AgI: Insoluble in water

III. Major Uses or Sources

Silver metal (Ag) and silver compounds are used in a wide variety of ways. In the past, silver was used for surgical prostheses, fungicides, and in coinage. Current uses include photographic materials, electrical and electronic products, silver paints, batteries, brazing alloys and solders, mirrors, sterling ware, jewelry, dental amalgams, burn ointments, as a catalyst in the manufacture of formaldehyde and ethylene oxide, in the purification/disinfection of drinking water and swimming pools, and in cloud seeding (Grayson, 1978; Smith and Carson, 1977). Silver halides (AgCl, AgI) are widely used in photography and cloud seeding (AgI), silver oxide (AgO) is used in making batteries, silver nitrate ($AgNO_3$) has the widest use of all Ag compounds. It is used in photography, as a starting agent for other Ag salts, in plating, for silvering mirrors and as a germicide and antiseptic (for newborns). Due to their bactericidal properties, silver complexes such as silver sulfadiazine are used in the treatment of burns.

Silver is released to the environment through natural processes such as erosion and through anthropogenic activities. Airborne sources of silver include: processing of ore, steel refining, cement manufacture, fossil fuel combustion, municipal waste incineration, and cloud seeding (silver iodide). The total US annual anthropogenic release of silver to the atmosphere from production processes and consumptive uses in 1978 was estimated at 77,700 kg (Scrow *et al.*, 1981). Scow *et al.* (1981) estimated that about fifty percent of the silver released into the atmosphere from industrial operations will be transported more than 100 km and will eventually be deposited by precipitation. Silver is found at levels above background in groundwater, surface water and soil near hazardous waste sites (silver has been detected at 100% of the 2,783 Superfund hazardous waste sites). Elevated levels of silver have been detected in shellfish near industrial/sewage inputs (Byrne & DeLeon, 1986; Pesch *et al.*, 1977), in the vicinity of smelters or mining operations, and in crops grown on sludge-amended soils. The intake of silver from dietary sources is estimated to range from 60-80 µg/day (Snyder *et al.*, 1975).

The major forms of silver in the atmosphere are probably metallic silver, silver sulfide, silver sulfate, silver carbonate, and silver halides (Smith and Carson, 1977). Background levels of silver appear to be less than 1.0 ng/m^3 (Davidson *et al.*, 1985), and it is estimated that most of the U.S. population breathes air containing a maximum of 1.0 ng/m^3 silver, which contributes a maximum of 0.023 ug silver/day (Letkiewicz *et al.*, 1984). The highest particulate levels of silver have been measured in the Coeur d'Alene River Basin in Idaho, where the greatest amount of silver is mined. Air levels of silver there ranged from 0.936 - 36.5 ng/m^3 with a mean of 10.5 ng/m^3 (Ragaini *et al.*, 1977).

IV. Effects of Human Exposure

According to the majority of literature reviewed, the sole clinical condition which results from chronic inhalation (or ingestion) exposure to silver salts or colloidal silver is the development of argyria; the localized or generalized impregnation of tissues with silver. A permanent gray or blue-gray discoloration may occur on the skin, conjunctiva, other mucous membranes, or internal organs, and is most pronounced in areas exposed to light. In their analysis of 357 cases of argyria, Hill and Pillsbury (1939) stated that, apart from aesthetic considerations, "argyria

produces no significant disturbance of the physiology of the affected organs or of the general health of the patient". Other investigators have maintained that deposition of silver affects many organs /tissues of the body, including lungs, kidney, CNS, and stomach.

Air concentrations of silver capable of producing argyria are not known with certainty. Data from the 1930's - 1970's from a large photographic manufacturing facility indicate that generalized argyria was seen when atmospheric levels were on the order of 1.0 mg Ag/m^3 (Stokinger, 1981). Levels estimated to be on the order of 0.1 mg/m^3 of silver resulted in some hyperpigmentation of the mucous membranes of the nose and throat and some ocular staining. No cases of argyria or other adverse effects were reported where average exposures were about 40-60 μg Ag/m^3 with values as high as about 150 μg Ag/m^3.

The concentrations of silver in body organs resulting in discoloration and/or clinical symptoms is not known. Hill and Pillsbury (1939) estimated that a total dose of 1-8 grams of silver would be required to induce argyria in a long-term inhalation exposure situation. Fowler and Norberg (1986) reported as 'normal' silver tissue concentrations ranging from <0.005-0.045 (kidney), <0.005-0.032 (liver) and <0.005-0.060 (lung) mg/kg wet wt in autopsy specimens taken from eight individuals living approximately 50 km from a metal smelter/refinery. Though causes of death were not specified by the study's authors, it is presumed that these individuals did not have argyria or other adverse health effects, as they comprised a control group for exposed smelter workers. Hamilton *et al.* (1972) reported concentrations of silver in human kidney, liver and lung to be 0.001 $\pm$ 0.002, 0.006 $\pm$ 0.002, and 0.002 $\pm$ 0.0001 mg/kg wet wt, respectively.

DiVincenzo *et al.* (1985) conducted biologic monitoring of workers engaged in the smelting and refining of silver. The 8-hour time-weighted average (TWA) exposure to silver over a 2 month monitoring period ranged from 1-100 $\mu g/m^3$. Since silver is eliminated predominantly in the feces (more than 90%) (Fowler *et al.*, 1986), fecal excretion data was used to estimate body burdens of silver in exposed workers. Based on fecal excretion values for silver, the authors concluded that argyria is unlikely to occur in workers exposed to silver at either a TWA of 0.03 mg/m^3, or a body burden of 14 ug silver/kg of body weight. The annual body burden of silver for the population at large was estimated to be as low as 2 μg Ag/kg of body weight.

The U.S. EPA reference dose for chronic oral exposure (RfD) to silver is based on argyria as the critical effect (U.S. EPA, 1996). The critical human study used in the RfD determination is a report on 70 cases of generalized argyria following administration of organic and colloidal silver medication, including 13 cases of generalized argyria following intravenous (i.v.) silver arsphenamine injection therapy for the treatment of syphilis (Gaul and Staud, 1935). In the i.v. study, data is presented for 10 males and 2 females who were administered 31-100 i.v. injections of silver arsphenamine, corresponding to a total dose of 4-20g, over a period of 2 to 9.75 years. In some patients, argyria developed after a total dose of 4, 7, or 8 g silver arsphenamine, whereas in others, discoloration was not seen until a total of 10, 15, or 20 g had been injected. Aside from syphilis, no information on the patients health status was provided. The authors concluded that since the body accumulates silver throughout life, variations in individual susceptibility were due to differences in body burdens of silver in the patients. The lowest observed adverse effect level for this study was 0.9 g metallic silver, based on an i.v. dose of 4 g silver arsphenamine in one

patient. As part of the same study (Gaul and Staud, 1935), a biospectrometric analysis of skin biopsies from ten cases of argyria led the authors to conclude that argyria becomes clinically apparent after a total accumulated i.v. dose of approximately 8 g of silver arsphenamine, which is approximately equivalent to 2 g of metallic silver.

Aside from silver-containing medications, which have largely been replaced by antibiotics, silver salts, particularly silver nitrate, are the compounds most frequently associated with the development of argyria, as well as other adverse health effects. Two men with argyria of the respiratory tract, who were employed in the manufacture of silver nitrate, had symptoms of mild chronic bronchitis (Montaudon 1959). Bronchoscopy revealed plaques of pigmentation along the trachea and around the orifices of the smaller bronchi. Bronchial and nasal mucosa also had silver particles deposited in the basement membrane, along with some phagocytosis and squamous metaplasia. No information on air concentrations, exposure duration, or past exposures was available.

Several investigators have described CNS effects ranging from paralysis, loss of coordination, and convulsions in argyric patients following exposure to silver compounds (Dreisbach, 1974; Reinhardt *et al.*, 1971; Rosenman *et al.*, 1979). Renal arteriosclerosis has also been reported in argyric patients with silver pigmentation of the kidneys (Gettler *et al.*, 1927; Kino, 1909). Zech *et al.* (1973) described a case of nephrosis in a 73-year old man with generalized argyria and silver deposits in the glomrular basement membranes who had used silver-containing mouthwash for 10 years. His estimated cumulative dose of metallic silver was 88 g. Marshall and Schneider (1977) have described an argyric patient with topical exposure to silver nitrate who complained of abdominal pain. Gastroscopy revealed a blue-gray discoloration of the esophagus, stomach and duodenum.

No controlled epidemiological studies have been conducted in humans. Occupational studies suggest that respiratory irritation, impairment of vision, gastrointestinal distress, or renal histopathology may result from chronic exposure to silver in humans. The principal route of exposure in the occupational studies is believed to be inhalation, but the possibility of some oral or dermal exposure cannot be excluded.

Rosenman *et al.* (1979) conducted a clinical field study of a group of 30 workers involved in the manufacture of silver nitrate and silver oxide. Six workers were found to have argyria and 20 had argyrosis (ocular deposition of silver). A number of other clinical symptoms were reported, including abdominal pain, renal insufficiency, decreased creatinine clearance, epistaxis, and decreased night vision. The occurrence of abdominal pain is thought possibly to be related to the corrosivity of the silver salts rather than a systemic effect of silver. Complaints of decreased night vision in 10 of the workers was associated both with duration of employment, and deposition of silver in conjunctiva or cornea. Time weighted averages of silver at the production plant, taken 4 months prior to the medical examinations, were in excess of established TLVs, and ranged from 0.039 - 0.378 mg/m^3. Unfortunately, individual exposures could not be quantified from the data presented. Duration of employment ranged from 10 months to 25 years with a median of 5 years. No skin discoloration was seen in workers with an employment history less than 5 years.

In a follow-up study of the same cohort of workers studied by Rosenman *et al.* (1979), Moss *et al.* (1979) conducted electrophysiologic and psychophysiologic testing of the eyes of 7 of the 10 workers who had complained of decreased night vision. Although all 7 workers had extensive pigmentary deposition, and ocular argyrosis was frequently present where occupational exposure was less than one year, no functional deficits were found.

Similar complaints were recorded for 27 workers engaged in the manufacture of silver ingots and silver metal powders (Rosenman *et al.*, 1987). Numerous chemicals including nitric acid, hydrochloric acid, formaldehyde, caustics, hydroquinone and solvents were used in the process. The average working life of the subjects was 8.1 years. Air levels at the time of the study were not measured, but previous air sampling conducted at the plant found that time weighted exposures to silver ranged from 0.04 - 0.35 mg/m^3. Fifty-six percent (15 of 27) of the workers evaluated complained of itchy, red, or watery eyes, sneezing, runny or stuffy nose, or sore throat. Nine workers reported lower respiratory symptoms of coughing, wheezing, or tightness in the chest. Eight of the 27 complained of nose bleeds. Six of the exposed workers reported decreased night vision. Although not statistically significant, individuals with corneal opacities were 3-5 times more likely to report night vision difficulties than those without silver corneal deposition.

Kidney function analyses of the exposed workers revealed increased urinary enzyme N-acetyl-B-D glucosaminidase (NAG) levels and decreased creatinine clearance relative to the controls. However, concomitant exposure to cadmium, an established nephrotoxin, in the silver exposed individuals precludes any conclusions being drawn about the renal effects of silver from this study.

In a study by Pifer *et al.* (1989), silver reclamation workers chronically exposed to insoluble silver halides (primarily silver bromide) exhibited a marginal decrease in red blood cell count and a significant increase in mean corpuscular volume relative to the controls (number not specified). The toxicological significance of these findings is unclear. Argyrosis was present in 7 of the 27 workers, but was not associated with any visual impairment. Annual 8 hour exposure rate estimates for the major recovery occupations ranged from 30-200 μg silver/m^3. Biological monitoring of 18 exposed workers revealed a mean fecal silver concentration of 16.8 mg/kg. No abnormalities were observed in chest radiographs or pulmonary function tests.

Forycki *et al.* (1983) reported on a case of lung damage with pulmonary edema in a worker exposed to heated vapors of metallic silver. The exposure took place over a 4 hour period in a small, enclosed room with no ventilation. The concentration of the silver vapors in the work room air was not known. The worker first became ill at 14 hours post exposure. Initial symptoms consisted of a rapid pulse, low oxygen content of the capillary blood, and a scattered thickening of the lungs observed in chest radiograms. The patient's condition progressed to acute respiratory failure, from which the patient eventually "recovered fully". No information on the length of the follow-up period nor of the occupational history of the worker prior to this incident was available.

Penetration of silver through the human placenta was demonstrated in a woman who was fatally intoxicated with silver nitrate (Reinhardt *et al.*, 1971). Silver was detected in the liver, lung, and muscle tissue of the fetus, but not in the brain.

Although historical evidence suggests that deposition of silver in the body causes little or no pathophysiological effects, a number of studies have suggested that the binding of silver to membrane and nucleus may affect enzymatic functions as well as other biochemical processes. Silver has been shown to inhibit the oxidation of glucose, glycerol, fumarate, succinate and lactate (Williams *et al.*, 1989). Silver decreases the activities of lactate dehydrogenase (Williams *et al.*, 1989; Menon and Wright, 1989) and glutathione peroxidase (Wagner *et al.*, 1975), and induces peroxidation of membrane lipids in liver (Rungby, 1990), which has been shown to interfere with several free-radical scavenging mechanisms. Silver salts have been shown to cause a sharp decrease of the copper concentration in serum (Pribyl *et al.*, 1982, 1989), and this deficiency has been linked to severe alterations of embryogenesis in animal studies (Shavlovski *et al.*, 1995). Copper is also thought to be necessary for normal functioning of the immune system (Koller, 1980), and silver has been shown to elicit autoimmune responses in experimental animals (Hultman *et al.*, 1994a, 1994b), and is cytotoxic to human immune cells *in vitro* (Steffensen *et al.*, 1994).

V. Effects of Animal Exposure

No animal studies following chronic inhalation exposure to silver or silver compounds were found. Konradova (1968) observed ultrastructural damage and disruption of cells of the tracheal epithelium of rabbits following an acute inhalation (2-8 hours) of an aerosol containing colloidal silver (10% silver).

Repeated exposure of animals to silver compounds (primarily silver salts) may result in anemia, cardiac enlargement, growth retardation, degenerative changes in the liver and kidney, and immunological perturbations.

Olcott (1950) demonstrated hypertrophy of the left ventricle of the heart in a statistically significant number of rats following long-term administration of silver nitrate or silver chloride in drinking water. Enlargement of the left ventricle was seen at a dose of 88.9 mg silver/kg/day. Left ventricle size increased with exposure duration, and showed a tendency to increase with dose of silver. A few, scattered granules of silver were found in the heart. Pigmentation was also present in the thyroid gland, adrenal gland, brain, and eye, and was most pronounced in the portal vein, hepatic histocytes and kidney. The number of animals with advanced pigmentation was not specified. The authors hypothesized that structural changes in the basement membranes of the renal glomeruli may have exerted some influence on the myocardium, as has been observed in other disease states.

Rats given a 0.25 % solution of silver nitrate for up to 37 weeks in their drinking water experienced reductions in body weight gain, ocular argyria and elevated mortality beginning 23 weeks after their initial exposure (Matuk *et al.*, 1981). Unfortunately, no information on water

consumption was provided, and since rats have been shown to decrease their water intake precipitously beginning on the 1st day of exposure to silver nitrate in drinking water (Walker, 1971), no conclusions about the effects of $AgNO_3$ on growth retardation or premature mortality can be drawn from this study.

Shouse and Whipple (1931) demonstrated hyperplasia of the bone marrow in dogs following a single intravenous injection of 500 mg colloidal silver (85.87% metallic silver). Liver and spleen contained the greatest amounts of silver. Repeat injections of smaller amounts of silver (200-300 mg) resulted in anemia, which the authors believe to be due to the hemolysis resulting from the trauma associated with the treatment protocol.

Fuchs and Franz (1971) observed deposits of silver granules in the glomeruli and tubules of the kidney as well as pathological changes in the renal tubules of rats exposed to 0.2 % silver nitrate in drinking water from 10-50 weeks.

A number of investigators have shown that silver administration to vitamin E and/or selenium deficient rats significantly increases the development of hepatic necrosis (Shaver and Mason, 1951; Grasso *et al.*, 1970; Wagner *et al.*, 1975). This toxicity is thought by some to result from inhibition of the selenoenzyme glutathione peroxidase (GSH-px).

Female SJL mice treated with 0.05% or 0.01% silver nitrate in drinking water for 5 weeks developed autoimmune conditions, including the production of high titers of IgG anti-nucleolar antibodies (ANoA), with the protein fibrillarin as the major target (Hultman *et al.*, 1994). There were no overt signs of illness in the exposed mice. Kidney, spleen and liver accumulated a substantial dose of the ingested silver. Although the significance of ANoA induction is not known, reactivity with fibrillarin has been observed in patients with scleroderma and anti-fibrillarin antibodies (Reimer *et al.*, 1988).

Rungby and Danscher (1984) conducted open field testing in mice following long term administration (125 days) of low, subacute doses of silver nitrate (0.015%) in drinking water. The finding that levels of activity were significantly lowered throughout the test period suggests that accumulations of silver in CNS might influence the function of the mammalian brain. Silver deposition was highest in certain areas of the brain involved in motor control. No overt signs of illness or decreases in weight were observed in the silver treated mice.

The effects of dietary administration of silver salts upon embryogenesis in rats was studied (Shavlovski *et al.*, 1995). Administration of 50 mg AgCl/day throughout gestational days 1-20 resulted in post-implantational death of embryos, external abnormalities, including omphalocele, eventration and shortened tail, and a significant reduction in fetal body mass. Enzymatically active copper-containing ceruloplasmin (CP) was found to be eliminated from the blood plasma of the dams and copper content in placenta and embryonic tissues dropped to almost zero. Superoxide dismutase activity in female and embryonic tissues was also decreased relative to controls. No embryotoxicity was observed in the group fed 50 mg AgCl/day from day 7-15. Embryotoxicity was reduced in dams injected i.p. with CP. The authors hypothesize that the embryotoxic effect of AgCl is due to its ability to interfere with copper metabolism, since

embryogenesis requires an enhanced transport of copper towards the growing tissues (Dokumov, 1968).

Neuronal accumulation of silver has been demonstrated in brain tissue of progeny from argyric rats (Rungby and Danscher, 1983a). Pregnant dams were exposed i.p. to silver lactate on gestational days 18 and 19. The amount and distribution of silver in the CNS of new-born rats resembled that seen previously in adult rats exposed to silver lactate (Rungby and Danscher, 1983b). No malformations or dysfunctions were observed in the offspring followed through postnatal day 45, although no information on specific endpoints examined was provided. According to the authors, the question of whether more discrete dysfunctions might arise from the presence of silver in growing or mature neurons remains to be settled. Silver seems to have a longer half-life in intracranial structures than in other organs (Furchner *et al.*, 1968).

VI. Derivation of Reference Dose (RfD)

Study	Gaul and Staud, 1935; Spiegel, 1931 (evaluated by U.S. EPA, 1996)
Study population	10 men (23-64 years), and 2 women (23 and 49 years)
Exposure method	Human therapeutic i.v. exposure over 2-9 years
Critical Effect	Argyria
LOAEL	0.9 g (total i.v. dose)
NOAEL	Not observed
Exposure continuity	31 i.v. injections
Exposure duration	2 years
LOAEL uncertainty factor	1 (Cosmetic effect without associated adverse health effects)
Subchronic uncertainty factor	1
Interspecies uncertainty factor	1
Intraspecies uncertainty factor	3 (effect in 1 sensitive individual only)
Cumulative uncertainty factor	3
Oral reference exposure level	0.005 mg/kg/day (U.S. EPA RfD)
Route-to-route extrapolation factor	3,500 μg Ag/m^3 per mg/kg/day
Inhalation reference exposure level	20 μg Ag/m^3

In their report on 70 cases of argyria (Gaul and Staud, 1935), data is presented for 12 individuals who developed argyria following administration of i.v. injections of silver arsphenamine over a 2 to 9.75 year period. Since the lowest i.v. dose resulting in argyria in one patient over a 2 year period was 4 g silver arsphenamine, and the fraction of silver in silver arsphenamine is 0.23, the LOAEL for this study was 0.9 g metallic silver. A biospectrometric analysis of skin biopsies from 10 cases of generalized argyria revealed that argyria becomes clinically apparent after a silver retention approximating an equivalent of 8 g silver arsphenamine. These findings are in agreement with other investigators. However, since the body accumulates silver throughout life, it is possible that the case in which argyria appeared after a total i.v. dose of 4 g silver arsphenamine may have had an existing body burden of silver prior to initiation of treatment.

Human and animal experimental data indicate that as much as 1-18% of the silver absorbed may be retained by the body (Furchner *et al.*, 1968; Newton and Holmes, 1966; East *et al.*, 1980). In a case of accidental inhalation of radioactive silver dust , 25% of the inhaled material accumulated in the liver between 2-6 days post exposure (Newton and Holmes, 1966).

The liver has been shown to be the major site of silver deposition and, at least in dogs, the build-up of silver in this organ is more gradual following inhalation than i.v. administration (Phalen and Morrow, 1973). Similar clearance values have been obtained for liver following human exposure to radioactive silver either by inhalation or i.v. administration (Newton and Holmes, 1966; Polachek *et al.*, 1960). Results from two human inhalation accidents, both involving airborne ^{110m} Ag, show that about 80% of the deposited material was cleared with a biological half-life of approximately 1 day. The remainder cleared with a half-life in the liver of 43 days in one case and 15 days in the other case (Newton and Holmes, 1966). In a case of i.v. administration of ^{110m} Ag, the biological half-life in the liver was 48 days. With the exception of brain and spleen, silver appears to clear from the whole body at the same rate as from the liver (Furchner *et al.*, 1968), and inhalation seems to lead to less exposure of the spleen to silver than is often seen in i.v. studies (Phalen and Morrow, 1973).

The chronic REL was derived from the Gaul and Staud study by selecting the LOAEL of 1 g metallic silver for the onset of argyria. The study is considered a subchronic exposure since the duration was for a period of 2 years. This is equivalent to a dose of 20 µg Ag/kg/day over the 2 years.

Since the LOAEL was derived from a sensitive individual in a subpopulation of persons of compromised health, and argyria has not been seen elsewhere at this exposure level, an uncertainty factor of 3 was applied for sensitive human subpopulations.

Based on a 25% retention upon inhalation and a 20 m^3/day respiratory volume, exposure to 0.7 µg Ag/m^3 over a lifetime of 70 years would result in a deposition of no more than 90 mg silver. For perspective, workers exposed to silver at the TLV of 0.1 mg/m^3 as a TWA over 40 years would be expected to retain as much as 2.4 g.

Medical examination data from the 1930's through 1978 from a large photographic manufacturing facility indicate that generalized argyria was seen when atmospheric levels were

on the order of 1.0 mg /m^3 silver (Stokinger, 1981). Levels estimated to be on the order of 0.1 mg/m^3 of silver resulted in some hyperpigmentation of the mucous membranes of the nose and throat and some eye discoloration. Since the 1930's, no cases of argyria or other adverse effects have been reported where average exposures were about 40-60 μg Ag/m^3 with values as high as about 150 μg Ag/m^3. If 60 μg Ag/m^3 is taken as the NOAEL, and adjusted for 24/day 7 day/week exposure, and uncertainty factors of 10 for sensitive human subpopulations and 10 for less than lifetime exposures are applied, the inhalation REL is 0.1 μg Ag/m^3. However, due to the lack of specific information on exposure concentrations and durations, the Gaul and Staud (1935) study is recommended for the derivation of the chronic reference exposure level for silver.

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CHRONIC TOXICITY SUMMARY

SODIUM HYDROXIDE

(caustic soda; caustic flake; white caustic; soda lye; sodium hydrate)

CAS Registry Number: 1310-93-2

I. Chronic Toxicity Summary

Inhalation reference exposure level	**2 µg/m^3**
Critical effect(s)	Moderate to severe irritation of the eyes, skin, and respiratory system in humans
Hazard index target(s)	Respiratory system; eyes

II. Chemical Property Summary (HSDB, 1995)

Molecular formula	NaOH
Molecular weight	40.01 g/mol
Description	Colorless to white flakes
Vapor pressure	1 mm Hg @ 739°C
Solubility	Soluble in water, alcohol and glycerin
Conversion factor	Not applicable (dust)

III. Major Uses and Sources

Sodium hydroxide is a strong base used in many industrial processes including the manufacture of chemicals, rayon, soap and detergents, pulp and paper, petroleum products, cellophane, textiles, explosives, and batteries (HSDB, 1995; ACGIH, 1992). Processes requiring sodium hydroxide also include etching, electroplating, and metal descaling.

IV. Effects of Human Exposure

Mortality was examined among 291 workers occupationally exposed to caustic dust up to 30 years after their initial exposure (Ott *et al.*, 1977). Workers were exposed to sodium hydroxide concentrations estimated to range from 0.5 to 2.0 mg/m^3 8-hour time weighted average (TWA) in different production areas of a chemical operations plant in which flakes or beads of concentrated sodium hydroxide were formed from a chlorine cell effluent. A survey of visits to the medical department from 1954-1972 showed employees reported symptoms of nasal, skin, and to a lesser extent, respiratory irritation. Responses were rated as "mild" if transient irritation occurred with or without erythema, and "moderate to severe" if objective damage to the exposed organ

occurred. Total office visits in which a body system was affected from sodium hydroxide exposure averaged 12.3 and 19.9 visits per 100 person years in 2 different production areas where exposure occurred. No significant change in worker mortality was found.

A case history was reported of a 63-year-old man who was exposed daily for 20 years to sodium hydroxide without protective equipment from using boiling lye as a cleaning solution (Rubin *et al.*, 1992). The patient had never smoked. Pulmonary function tests, x-ray of the chest and evaluation of blood gases suggested severe obstructive airway disease with significant air trapping. Levels of exposure were not quantitated.

A 25-year-old woman exposed for one day (~ 8 hours) to caustic soda in the treatment of wood developed irreversible corrosive lung injury (reduced FEV_1, total lung capacity) which was observed both 10 weeks and one year after the initial exposure (Hansen and Isager, 1991). Initially, the patient was hospitalized with bronchopneumonia and severe laryngitis. Dyspnea was reported by the patient 1 year after the injury. Exposure levels were not reported.

Workers exposed to 0.01 to 0.7 mg/m^3 heated sodium hydroxide along with several other solvents including benzene, n-butyl acetate, Stoddard solvent, and sulfuric acid reported burning and redness of the nose, throat and eyes (Hervin and Cohen, 1973).

V. Effects of Animal Exposure

The chronic toxicity of sodium hydroxide from inhalation exposure has not been studied extensively in animals. Rats were exposed by inhalation to an unknown concentration of sodium hydroxide produced from an aerosolized 40% solution for 30 minutes twice daily for 2.5 months (Dluhos *et al.*, 1969). Examination of the lung revealed alveolar wall thickening with cell proliferation accompanied by congestion. Ulceration and flattening of the bronchial epithelium and proliferation of the lymphadenoid tissue were also noted.

Twenty-seven rats were exposed twice weekly for one month to an aerosol produced from a 40% sodium hydroxide solution (Vyskocil *et al.*, 1966). All the rats died, predominantly from broncho-pneumonia. Exposure to an aerosol produced from a 20% sodium hydroxide solution resulted in dilatation and destruction of alveolar septae. Bronchial dilation and mucus membrane degeneration were observed in animals treated with an aerosol of 5% sodium hydroxide solution, however, a group similarly treated with a 10% sodium hydroxide solution did not show adverse effects.

VI. Derivation of Chronic Reference Exposure Level (REL)

Study	Ott *et al.*, 1977
Study population	Occupationally exposed humans (291)
Exposure method	Occupational inhalation exposures
Critical effects	Moderate to severe irritation of the eyes, skin, and respiratory system
LOAEL	0.5 mg/m^3 (low estimate in range)
NOAEL	Not observed
Exposure continuity	8 hr/day (10 m^3/day occupational inhalation rate), 5 days/week
Exposure duration	Up to 30 years
Average occupational exposure	0.18 mg/m^3 for LOAEL group
Human equivalent concentration	0.18 mg/m^3 for LOAEL group (particulate with respiratory effects)
LOAEL uncertainty factor	10
Subchronic uncertainty factor	1
Interspecies uncertainty factor	1
Intraspecies uncertainty factor	10
Cumulative uncertainty factor	100
Reference exposure level	0.002 mg/m^3 (2 µg/m^3)

The Ott *et al.* (1977) study was selected for the development of the chronic reference exposure level because it demonstrated an adverse health effect in humans exposed to sodium hydroxide chronically and included quantitation of exposure levels. Reports on the toxicity of sodium hydroxide indicate its effects are fairly non-specific and toxicity tends to occur at sites in direct contact with the compound. Although there is no evidence that the toxicity of sodium hydroxide is cumulative, the presence of "objective damage" to exposed organs at the lowest reported levels of exposure suggests this level should be taken as a LOAEL for chronic exposures. The Ott *et al.* (1977) study, which included evidence of respiratory irritation among workers occupationally exposed to sodium hydroxide, is consistent with the case report of respiratory damage from long-term exposure to sodium hydroxide reported by Rubin *et al.* (1992). Furthermore, changes observed in the tissues of the lung and respiratory system of animals exposed to sodium hydroxide for up to 2.5 months also suggest that the irritation of the respiratory system in humans is a likely target of sodium hydroxide's toxic action (Dluhos *et al.*, 1969). No studies, however, clearly demonstrated a dose-response relationship between exposure level and toxicity.

The strengths of the inhalation REL include the use of human exposure data from workers exposed over a period of years. Major areas of uncertainty are the uncertainty in estimating exposure andthe potential variability in exposure concentration, the lack of reproductive and developmental toxicity studies, and the lack of observation of a NOAEL.

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CHRONIC TOXICITY SUMMARY

STYRENE

(ethenylbenzene, phenylethylene, vinylbenzene)

CAS Registry Number: 100-42-5

I. Chronic Toxicity Summary

Inhalation reference exposure level	**1,000 μg/m^3**
Critical effects(s)	Neurotoxicity in humans
Hazard index target(s)	Nervous system

II. Chemical Property Summary

Molecular formula	C_8H_8
Molecular weight	104.16
Description	Colorless to slightly yellow liquid
Vapor pressure	10 mm at 31^{o}C, polymerizes at 82^{o}C and above (Weast, 1979)
Solubility	310 μg/ml (Dean, 1985)
Conversion factor	4.26 μg/m^3 per ppb at 25^{o}C

III. Major Uses and Sources

The major source of styrene is industrial synthesis in which ethylbenzene is the starting material (ATSDR, 1992). The major uses of styrene are in polystyrene manufacturing, the butadiene-styrene rubber industry, and in the reinforced plastics industry (RPI) (WHO, 1983). Major non-styrene contaminants in the butadiene-styrene rubber industry are butadiene, benzene, carbon disulfide, and trichloroethylene, whereas the main co-contaminants associated with the RPI are glass fibers and acetone (WHO, 1983). Environmental exposures to styrene may result from mainstream cigarette smoke (Newhook and Caldwell, 1993) and newly installed carpets containing a styrene-butadiene rubber latex adhesive (Hodgson *et al.*, 1993). The Third National Health and Nutrition Examination Survey (Ashley *et al.*, 1994) reported a mean blood styrene level among ≥ 600 individuals as 0.074 ppb.

IV. Effects of Human Exposure

Chronic exposures to styrene (to be discussed below) result in central nervous system (CNS) and peripheral nervous system effects, although the latter are not as pronounced (ATSDR, 1992; Rebert and Hall, 1994; Murata *et al.*, 1991). The evidence for styrene induced hepatic changes is either negative or equivocal (ATSDR, 1992). Although renal changes in workers exposed to 212 ppm (8-hour workday) are suggested by increased urinary enzymes, exposure of another workforce to 53 ppm did not result in the urinary excretion of other proteins. Some human studies suggest chronic exposure to styrene results in reproductive effects, but the limited data are difficult to interpret because of the small sample numbers (Brown, 1991; Lindbohm, 1993). Immunologic alterations (e.g., altered phenotypic profiles among lymphocyte subsets, decreased natural killer cell activity, and decreased chemotaxis) have also been observed, but the limited data prevent quantitative interpretation (Bergamaschi *et al.*, 1995; Governa *et al.*, 1994).

Unlike the CNS depressant effects of acute exposures to high styrene levels, which are probably mediated by the direct effect of the lipophilic, unmetabolized styrene on nerve cell membranes, long-term effects of styrene exposure may result from the action of one or more metabolites of styrene (Savolainen, 1977; Mutti *et al.*, 1988). In humans, styrene metabolism is initiated by cytochrome P450 (P450)-mediated oxidation of styrene to a reactive metabolite, styrene oxide (SO). The reaction takes place in human liver and, to a minor extent, in lung (Nakajuma *et al.*, 1994). The P450 enzymes responsible for the epoxidation of styrene to SO are also found in human brain, but the brain isozymes have not been tested specifically with styrene as substrate (Bhamre *et al.*, 1993). Styrene may also be oxidized to SO by enzymes which share specific iron and porphyrin components with P450 and those that utilize active oxygen species (Belvedere *et al.*, 1983; Tursi *et al.*, 1983; Miller *et al.*, 1992).

The major end-product of styrene metabolism in humans is urinary mandelic acid (MA) and phenylglyoxylic acid (PGA) (Bardodej and Bardodejova, 1970; Leibman, 1975; Guillemin and Bauer, 1979). Other pathways that may be present in other animals are either absent or are quantitatively negligible in humans, except when high styrene levels are encountered (Guillemin and Berode, 1979; Chakrabarti *et al.*, 1993; Hallier *et al.*, 1995). Confounders of the quantitative relationship between styrene exposure and urinary MA+PGA are the consumption of ethanol (Berode *et al.*, 1986) and exposure to ethylbenzene (Bardodej and Bardodejova, 1970). An important consequence of ethanol related decreased levels of urinary MA is the potential underestimation of exposure to styrene (Guillemin and Bauer, 1979; Berode *et al.*, 1986). However, the urinary metabolite levels return to control values 4-5 hours after the ethanol consumption (Berode *et al.*, 1986).

Indicators of human styrene exposure include exhaled styrene, blood styrene, urinary MA, and urinary MA+PGA (Guillemin and Berode, 1988). Exposure to styrene by inhalation results in 89 percent absorption (Guillemin and Berode, 1988). In the occupational studies that are the basis for quantifying the relationship between chronic styrene exposure and health effects, end-of-shift or next-morning MA+PGA have been used. The next-morning measurements are more reflective of past exposures due to the high fat solubility of styrene (fat:blood partition coefficient = 94 (Csanady *et al.*, 1994)), the presence of a second, long biological half-life for MA = 25 hours., and a long biological half-life for PGA = 11 hours (Guillemin and Bauer, 1979). Following

inhalation, the half-life for styrene is 41 minutes in blood (Wigaeus *et al.* 1983) and 32-46 hours in fat tissue (Perbellini *et al.*, 1988).

One postulated mechanism for the chronic non-cancer toxicity of styrene is the binding of the highly reactive SO to components of nervous tissue. Another postulated mechanism is an alteration in the levels of circulating catecholamines (e.g., dopamine) due to the binding of PGA to these biogenic amines (Mutti, 1993; Mutti *et al.*, 1984a; Checkoway, 1994) and the subsequent changes in physiological functions that are under biogenic amine control. Although long-term exposures to styrene are associated with decrements in physiological functions, the exact mechanism(s) for these effects have not been clearly established (see reviews by ATSDR, 1992; Mutti, 1993; Rebert and Hall, 1994).

Kolstad *et al.* (1995) estimated excess deaths due to four major non-malignant disease groups for 53,847 male workers in the Danish RPI. Low and high styrene exposures were based on companies with less than 50% (low) and those with 50% or more (high) employees involved with reinforced plastics. An internal comparison was made with workers unexposed to styrene to account for more similar activities and lifestyles. Statistically significant ($p < 0.05$) excess deaths due to pancreatitis and degenerative disorders of the myocardium and non-significant excess deaths due to degenerative diseases of the nervous system were observed. Non-significant excess deaths due to glomerulonephritis were also observed.

Checkoway *et al.* (1994) described a cross-sectional study of 59 male boat plant workers exposed to <1 to 144 (mean = 37.2) ppm styrene. Monoamine oxidase B (MAO-B) activity in platelets was measured as an indicator of catacholamine metabolism. When the styrene exposed workers were divided into quartile exposures, a dose dependent decrease in MAO-B activity was observed after adjustments were made for age, smoking, alcohol and medication use.

Female workers employed in the RPI were studied for levels of substances associated with neuroendocrine function (Mutti *et al.*, 1984a**).** Serum prolactin, thyroid stimulating hormone, human growth hormone, follicle stimulating hormone, and luteinizing hormone were measured in 30 women who were between the 5th and 15th day of the menstrual cycle. Exposure was based on the next-morning MA+PGA and levels of the neuroendocrine substances were measured In venous blood samples taken the next-morning before the start of work. On the basis of a relationship (not detailed in the report) between urinary metabolites and styrene air concentration, the authors estimated the average styrene TWA/8 hr was about 130 ppm. Controls consisted of women who were factory workers living in the same area as the styrene-exposed women, but who were not knowingly exposed to styrene. After controlling for age and exposure time, the increased prolactin and thyroid stimulating hormone levels were correlated with the concentration of next-morning urinary MA+PGA, although only the increased prolactin levels were statistically significant.

Disturbances to the CNS have been observed in occupational settings. Decreased manual dexterity and increased reaction times were observed by Mackay and Kelman (1986), Flodin *et al.* (1989), and Cherry and Gautrin (1990) for air styrene levels of 25 ppm to more than 100 ppm. However, in each of these studies, there were difficulties in quantifying the effect. The difficulties included small sample size, unknown exposure duration, and either unknown ethanol consumption or lack of adjustment for ethanol consumption. In the Cherry and Gautrin (1990)

investigation, however, the authors determined that ethanol consumption did not reduce the correlation between increased reaction time and exposure.

Decrements in other CNS functions were observed among workers in the well controlled studies of Fallas *et al.* (1992), Chia *et al.* (1994), and Mutti *et al.* (1984b). Fallas *et al.* (1992) studied 60 male workers (average age = 29.5 years, average air styrene = 24.3 ppm). The styrene-exposed population was compared to non-exposed worker controls and matched for age, intellectual level, and ethnic origin. The results from a standardized test battery showed decrements in the aiming response and 22/60 styrene exposed workers exhibited increased reaction times compared to 7/60 controls. Acquired color vision loss (dyschromatopsia) was also observed in the styrene-exposed workers compared to controls. Chia *et al.* (1994) also observed decrements in CNS function as defined by altered visual retention, audio-digit recognition, and digit recognition. However, a dose-response relationship did not exist. These workers also exhibited a statistically nonsignificant dose-dependent dyschromatopsia.

Dyschromatopsia among styrene workers in the RPI was also reported by Gobba and Cavalleri (1993) and Campagna *et al.* (1995). Workers (n=36) exposed to an average of 16 ppm styrene exhibited significantly greater dyschromatopsia than controls, matched for age ethanol consumption and tobacco smoking (Gobba and Cavalleri, 1993). Among the study population, only 1/36 styrene-exposed workers (compared to 16/36 controls) performed the test with 100 percent accuracy. When a different group of styrene-exposed workers was tested, those exposed to > 50 ppm air styrene exhibited greater dyschromatopsia than those exposed to ≤ 50 ppm, and within this group, a subset exhibited a similar decrement after returning from a one month vacation. In the Campagna *et al.* (1995) study, the test for dyschromatopsia was given to 81 RPI workers (79 male and 2 female) exposed to 4.6, 10.1, and 88.8 ppm styrene (first quartile, median, and third quartile, respectively). No control group was used in this study. Statistical analysis revealed a correlation of color vision loss with exposure to styrene (defined as next-morning urinary MA), age, and ethanol consumption.

Mutti *et al.* (1984b) studied a group of male styrene workers and a control group of manual workers exposed to styrene for an average of 8.6 years. Eligibility criteria included absence of metabolic, neurologic, or psychiatric disorders, limited ethanol intake, and limited cigarette usage. Styrene exposure was assessed by the next-morning urinary MA+PGA. Workers with metabolite concentrations of up to 150 mmoles/mole appeared to have no significant effects, and this level is therefore designated as the NOAEL in this study. The authors state that this level of urinary metabolites corresponds to a mean daily 8-hour exposure to air styrene of 25 ppm (106 mg/m^3). The 95% confidence interval was also calculated for an 8-hour exposure to 100 ppm, the lower limit of the confidence calculation being 88% of the mean styurene exposure. This factor was applied directly to the NOAEL of 25 ppm [25 ppm x 0.88 = 22 ppm (94 mg/m^3)]. A battery of neuropsychological tests designed to measure CNS function was given on the morning after the last work day in the week. In addition to matching for age, sex, and educational level, a vocabulary test was included to match for general intelligence. The tests assessed memory and sensory/motor function. The results were expressed as continuous and quantal data. For the continuous data, the degree of abnormal response to each of the eight tests was recorded.

Mutti *et al*. (1984b) expressed the quantal data as the fraction of tested subjects who responded abnormally to ≥ 1, ≥ 2, and ≥ 3 tests (see Table 1). When the quantal data were assessed by an analysis of probabilities of proportions (Kirby, 1993), the low dose group was significantly higher than controls (p=0.001). In addition, a three-way representation of the data (prevalence (number of respondents for at least one, two or three abnormal tests), duration (years at work), and intensity (metabolite level)) as presented in Mutti *et al.* (1984b), revealed an effect of duration as well as intensity. This study was well designed and executed in terms of experimental protocol and statistical evaluation, which included tests for false positive and false negative responses. While not all confounders (e.g., compensatory mechanisms, biorhythms, workers who leave because of styrene related illness) could be ruled out, this study does show that workers exposed to styrene over a long period of time show evidence of CNS dysfunction.

Table 1. Subjects Classified Positive on Neuropsychological Tests as a Function of Styrene Exposure [a].

MA+PGA (mmoles per mole creatinine [b]	Total Subjects	Number of Abnormal Tests		
		≥ 1	≥ 2	≥ 3
Controls	50	4/50	2/50	0/50
< 150 (mean = 75 ± 33)	14	6/14	3/14	2/14
150-299 (mean=216 ± 45)	9	6/9	5/9	3/9
300 - 450 (mean=367 ± 49)	14	10/14	7/14	5/14
> 450 (mean=571 ± 108)	13	11/13	8/13	6/13

[a] Data from Table IV in Mutti *et al.* (1984b).
[b] "Next-morning" urinary metabolites.

Exposure to styrene may affect the peripheral nervous system (PNS). In a case report (Bahari *et al.*, 1986), a man working for 5 years with a photostat process that utilized styrene was diagnosed with peripheral neuropathy. However, in occupational studies, the relationship between exposure to styrene and PNS effects have been inconsistent (Triebig *et al.*, 1985; Cherry and Gautrin, 1990). A major difficulty in understanding the potential for this relationship is the lack of knowledge about the appropriate surrogate for dose that leads to PNS disturbance (Murata *et al.*, 1991). In one study, however, chronic exposure indices were developed which included work method, years at work, time spent laminating (source of high exposure), styrene air concentration, and end-of-shift urinary MA (Matikainen *et al.* (1993). Numbness in the extremities increased with the exposure index, although the effect was statistically marginally insignificant ($p < 0.1$). The styrene TWA/8 hr was 32 ppm for the 100 study subjects.

Female reproductive toxicity has been inconsistently reported among humans (Brown, 1991; Lindbohm, 1993). These studies are difficult to interpret because of the high background rates of endpoints such as spontaneous abortion and menstrual disorders in combination with confounding exposures. In those studies which showed no reproductive effects due to styrene exposure, the power of the studies was low due to the small numbers of women. Hence the evidence for any adverse effects of exposure to styrene on female reproductive function is inconclusive.

Immune system alterations were reported in a study conducted by Bergamaschi *et al.* (1995). RPI workers (n=32 female/39 male, average age = 32 years, average exposure duration = 7 years, tobacco consumption = 7 cigarettes/day, ethanol consumption = 16 glasses/week) were compared with non-styrene exposed factory workers and matched for age, sex, tobacco use and ethanol consumption. Air styrene levels, among the different factories, varied between 10 - 50 ppm, and individual worker exposure was measured by urinary metabolites the morning after the last shift (15 hours post-exposure). Among all workers in the study (median exposure = 16 ppm - according to the data of Guillemin *et al.* (1982)), the proportion of 12/18 lymphocyte subsets and the prevalence of abnormal values of immunologic phenotypes for 11/18 subsets were statistically different from the controls ($p < 0.001$ to < 0.05). When the workers were placed into three exposure groups (0, < 25 ppm, and > 25 ppm styrene), dose-response relationships were observed for prevalences of abnormal responses for four lymphocyte subsets and in the case of two subsets, abnormal responses were observed in the group exposed to < 25 ppm styrene. Natural killer cell activity (a lymphocyte function) measured in a different group of workers in the same study) was decreased compared to unexposed worker controls. The median exposure was given in terms of urinary metabolites which was calculated as 21 ppm based on the data of Guillemin *et al.* (1982). The data show exposure of these workers to air styrene levels below 50 ppm, and probably at levels near 25 ppm, resulted in alterations of the immune system.

Governa *et al.* (1994) observed reduced chemotactic responses of polymorphonuclear lymphocytes (PMNs) obtained from 21 styrene exposed workers. However, the lack of exposure data prevents a quantitative assessment. In the same study, 0.1 - 0.6 mM styrene inhibited the chemotaxis of isolated healthy PMNs.

V. Effects of Animal Exposure

In a subchronic study, carried out under the auspices of NTP (NTP, 1992), mice and rats were exposed by inhalation to styrene vapors to establish a maximum tolerated dose for chronic studies. Mice were exposed to 0, 62.5, 125, 250, or 500 ppm styrene (6 hr/d, 5 d/wk, 13 wks). Among males early deaths occurred in the 250 ppm group. Body weights among all exposed mice were lower than controls, and the difference was about 9 percent. Lung, olfactory epithelial, and forestomach lesions were observed in females and males. In females, degeneration of the adrenal gland cortex was observed. An effect not discussed in the chairperson's report, but recorded in the original laboratory report, was an increased estrous cycle length among the female mice at all styrene doses. A LOAEL = 62.5 ppm is indicated by the olfactory epithelial, forestomach and respiratory tract lesions in mice of both sexes and for lesions in the adrenal cortex in the female mice. Rats were exposed to 0, 125, 250, 500, 1000, or 1500 ppm styrene (6 hr/d, 5 d/wk, 13 wks). No deaths occurred, but reduced body weights were observed at the two highest doses. Lesions of the respiratory tract were observed at all dose levels. A LOAEL = 125 ppm is therefore indicated for the rats. Rats were exposed by ingestion for 2-years to styrene in drinking water (0, 125, and 250 ppm) (water solubility of styrene is 310 ppm). The only effect was a styrene-related reduction in water consumption (Beliles *et al.*, 1985).

Kishi *et al.* (1995) carried out a developmental study on rat pups born to dams exposed by inhalation to styrene (0, 50, 300 ppm; 6-hr/d; gestation days 7-21). Although the small number of litters (n=2) at the 50 ppm dose prevented detailed statistical analysis, the data suggest that exposure of the dams to 50 ppm styrene resulted in deficits and delays in some motor and coordination abilities among the pups. Pups born to dams exposed to 300 ppm exhibited statistically significant increases in spontaneous activity and in the delay of some neurobehavioral functions. Many of the effects became diminished as the pups aged. Measurements of reproductive toxicity (maternal weight gain, length of gestation, number of live births) did not change. Postnatal body weights were lower among the styrene exposed pups, but the differences became less as the pups aged to 125-days.

Mice, exposed acutely (14 days) by inhalation to 125 - 500 ppm styrene exhibited decreased spleen / body weight, splenic hypocellularity, altered lymphocyte proportions among subsets, and increased proliferative response to mitogens (Corsini *et al.*, 1994). Mice and rats, exposed by gavage to high levels of styrene (18, 27, 45 mg/kg - mouse; 118, 177, 294 mg/kg - rat) for 5 days/week for 4 weeks, exhibited decreased resistance to encephalomyocarditis virus, *P. berghie* (a malaria parasite) and *N. braseleinisi* (a parasitic worm) (Dogra *et al.*, 1992).

VI. Derivation of the U.S. EPA Reference Concentration (RfC) (U.S. EPA, 1996)

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Study	Mutti *et al.* (1984b).
Study populations	Human (occupational)
Exposure method	Inhalation
Critical effects	Central Nervous System
LOAEL	
NOAEL	22 ppm
Exposure continuity	8 hr/d (10 m^3/day occupational inhalation rate), 5 d/wk
Exposure duration	8.6 years (average years at work)
Average occupational exposure	7.8 ppm for NOAEL group
Human equivalent concentration	7.8 ppm for NOAEL group
LOAEL uncertainty factor	1
Subchronic uncertainty factor	3
Interspecies uncertainty factor	1
Intraspecies uncertainty factor	3
Modifying factor	3
Cumulative uncertainty factor	30
Inhalation reference exposure level	0.03 ppm (300 ppb; 1 mg/m^3; 1,000 µg/m^3)

The study on which the U.S. EPA (1996) reference concentration (RfC) is based is well designed and executed. Careful attention was paid to include eligibility criteria for the control group that correct for confounders unique for this population, e.g., limited ethanol intake, a work-force not exposed to neurotoxic substances, and a test to allow a match for general intelligence. The use of urinary metabolites to measure exposure dose is based on the observation that the next-morning urinary MA+PGA is directly related to the air level of styrene. The Guillemin *et al.* (1982) RPI investigation provides the basis for the Mutti *et al.*, (1984b) conversion from urinary MA+PGA to styrene. For the calculation of the RfC, U.S. EPA (1996) used the 95 percent lower bound. The next-morning urinary metabolite level also emphasizes the chronic component of the exposure because of the observation that styrene and MA+PGA are not completely cleared between work shifts (Guillemin and Bauer, 1979; Perbellini *et al.*, 1988).

In the Mutti *et al.* (1984b) study, the results were presented as continuous and quantal data. The derivation of the U.S. EPA RfC for styrene (U.S. EPA, 1996) is based on the continuous data presented for each of the eight neuropsychological tests.

A confounder in the analysis of CNS disturbance among styrene-exposed humans is ethanol consumption. Although difficult to control in epidemiologic investigations, most studies either attempt to control for ethanol consumption or analyze the contribution of such behavior to the results. Also, the worker populations used for these studies represent many geographical locations, each with its own pattern of drinking behavior. The results of the aggregate of the studies reported in this summary show that exposure to styrene can result in CNS disturbance after accounting for ethanol consumption.

A major source of exposure uncertainty is the lack of exposure data on individual workers in the RPI. At the present time, a system does not exist to obtain such information, although a recent report suggests a methodology is being developed (Jensen *et al.*, 1995). The RPI, the industry from which the workers in the Mutti *et al.* (1984) study were taken, is characterized by a large turnover of highly exposed workers (Wong, 1990; Kogevinas *et al.*, 1993). Ethanol consumption may also interfere with exposure assessment based on biomonitoring due to the ethanol-related decreased levels of MA (Berode *et al.*, 1986). This effect is reversible, and should therefore be minimal if morning after samples are utilized.

The rat and mouse inhalation study (NTP, 1992) also contains data that may be used to develop a chronic exposure level for styrene. The mice were more sensitive to the styrene vapors than were rats and a LOAEL of 62.5 ppm was identified based on lesions in various organs in both sexes. The adjustment for discontinuous exposure is (6/24 x 5/7) = 0.18. The uncertainty factors are: 10 each for intraspecies variability, interspecies sensitivity, subchronic exposure, and adjustment for a NOAEL. The resultant exposure level = (62.5 ppm x 0.18) / 10,000 = 0.0011 ppm = 1.1 ppb (4.7 $\mu g / m^3$). While the exposure level based on the mouse data (NTP, 1992) is appropriate methodology, the RfC based on the well designed human study of Mutti *et al.* (1984) is preferable because it does not introduce uncertainties associated with interspecies and subchronic extrapolations.

The Agency for Toxic Substances and Disease Registry (ATSDR) calculated a chronic inhalation minimum risk level (MRL) for styrene (ATSDR, 1992). The calculation was based on the same Mutti *et al.* (1984b) worker study used by U.S. EPA to calculate the RfC. ATSDR (1992) identified the lowest exposure group as a LOAEL and assigned an air styrene level of 25 ppm. To derive the MRL, ATSDR corrected the LOAEL for discontinuous exposure and applied uncertainty factors (UFs) for the use of a LOAEL and for intraspecies variability. The MRL was calculated as: 25 x (8/24 x 5/7) / 10 x 10 = 0.06 ppm (ATSDR, 1992).

VII. Analysis of the U.S. EPA RfC for Styrene

The U.S. EPA (1996) calculated an RfC of 0.3 ppm ($1 mg/m^3$), and this value has been recommended by OEHHA, as the chronic REL. Two aspects of the RfC requires additional analysis. They are (1) the choice of an effective air styrene level (i.e. LOAEL or NOAEL and the assigned exposure level) and (2) the application of UFs.

Effective exposure level.

The data obtained by Mutti *et al.* (1984b) are presented in two forms. One form is continuous data in which the value of deviation from a normal response is measured as a function of exposure for each of eight individual tests. The other form is quantal data in which the proportions of subjects responding abnormally to ≥ 1, ≥ 2, and ≥ 3 tests are measured as a function of exposure. U.S. EPA (1996) used the continuous data and chose the short term logical memory tests that individually exhibited a dose-response effect. According to the data, no effect was observed at the lowest exposure level and this level was taken as the NOAEL. An abnormal response was observed for short-term verbal memory, but a dose-response was not apparent from the data. OEHHA staff also evaluated the quantal data and the Binomial Cumulative Function (Kirby, 1993) and determined the probabilities of abnormal responses among the exposed

subjects based on the unexposed subjects whose response was assumed to be normal. The probability of the proportion of subjects responding abnormally to the tests was $p \leq 0.001$.

NOAEL/LOAEL. The deviation from normal responses for each of the individual tests yields important information on the effect of styrene on a specific expression of central nervous system (CNS) function. However, individuals may express changes in altered CNS function in different ways. Such differential expression is expected given the complexity of the mechanism(s) of styrene-induced CNS toxicity that may involve styrene, styrene oxide (SO), or phenylglyoxylic acid (PGA) (Savolainen, 1977; Mutti *et al.*, 1988; Costa, 1996). In a population of 50 subjects, individual test-specific effects that occur at low doses may not have been observed. If the criterion for abnormality is expressed in terms of CNS dysfunction, defined by all tests, the sensitivity of the testing procedure is increased and the low dose effects are more easily observed. The quantal data of Mutti *et al.* (1984b), i.e. the proportion of subjects responding abnormally to the tests, therefore provides a more sensitive approach to detecting low dose effects. At the most restrictive criterion, i.e. the proportion of subjects responding abnormally (compared to matched controls) to ≥3 tests, the probability of a chance occurrence was ≤0.001.

Exposure level. The exposure data in the Mutti *et al.* (1984b) study were expressed quantitatively as the next-morning level of urinary metabolites (mandelic acid (MA) + phenylglyoxylic acid (PGA)). The numerical values represent mmoles MA+PGA / mole creatinine and these units will be understood in the discussion that follows. To assign the test subjects to each of four exposure levels, Mutti *et al.* (1984b) set boundaries such that level l = <150 units, level 2 = 150-299 units, level 3 = 300-450 units, and level 4 = >450 units (see Table 3 (Mutti *et al.*, 1984b)). In the analysis of individual test scores, U.S. EPA (1996) identified the lowest exposure group as the NOAEL and assigned an exposure level of 150 units, specifically "Workers with metabolite concentrations of *up to* 150 mmoles/mole appeared to have no significant effects, and this level is therefore designated as the NOAEL in this study." However, according to the data presented in Mutti *et al.* (1984b), the lowest exposure group presented urinary metabolites *less than* 150 units. The only information on the specific level of exposure to this group is summarized in Table 3 of Mutti *et al.* (1984b) and is presented as a mean exposure level, in this case 75. The mean level of urinary MA+PGA in the level 2 exposure group was 216. If the corrected exposure level is applied to the U.S. EPA analysis, the effective dose level will be decreased by half.

Uncertainty factors (UFs).

U.S. EPA (1996) applied three UFs, 3 for intraspecies variability, 3 to adjust a subchronic 8.6 year occupational exposure to a lifetime environmental exposure, and 3 to account for an inadequate data base. The choice of the UF for intraspecies variability of 3 depends on the interpretation of the use of the biological exposure index for styrene, i.e. urinary MA+PGA. A relationship between the next-morning urinary MA+PGA and air styrene level was developed for a worker population (Guillemin *et al.*, 1982), wherein the extrapolation was presented as a central value, an upper 95 percent- and a lower 95 percent confidence limit. U.S. EPA (1996) used the lower 95 percent limit (about 88 percent of the central value) and assumed that because this value takes into account differences in metabolism and toxicokinetic properties, only an intermediate UF of 3 is necessary to adjust for intraspecies variability. Important issues of

intraspecies variability may not be taken into account by this analysis. The exposure in the Guillemin *et al.* (1982) study, like the Mutti *et al.* (1984b) study, is an occupational exposure. Inherent in the use of occupational cohorts are two phenomena that could impact on the extrapolation of data from the styrene workers to the general population. They are (1) the selection of a healthy worker population at the time of entry into the industry, and (2) the survival of healthier workers after long periods of employment in the industry (Fox and Collier, 1976). Hence, the Guillemin *et al.* (1982) extrapolation does not take into account a population whose health status may be less than that of the studied worker population. A malnourished population may also be subject to a synergistic action of styrene on CNS function, as suggested by the effects of ingested styrene on rats fed low protein diets (Khanna *et al.*,1994). For these reasons, a UF of 10 to account for intraspecies variability may be justified. If the U.S. EPA RfC derivation were adjusted to reflect the use of an intraspecies variability UF of 10, the resulting RfC would be one-third of the current value, i.e. 0.26 / 3 = 0.089 = 0.09 ppm. If the adjustments to the effective exposure level were also made, the resulting RfC would be 0.035 ppm.

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CHRONIC TOXICITY SUMMARY

STYRENE-7,8-OXIDE

(Synonym: styrene oxide, styrene epoxide,1,2-epoxyethylbenzene, 2-phenyoxirane, epoxystyrene, phenylethylene oxide, phenethylene oxide)

CAS Registry Number: 96-09-3

I. Chemical Toxicity Summary

Inhalation reference exposure level	**6 μg/m^3**
Critical effects(s)	Lethality from respiratory effects (bronchitis, bronchiolitis) in rabbits; fetal resorptions
Hazard index target(s)	Respiratory system; reproductive system

II. Chemical Property Summary (HSDB, 1995)

Molecular formula	C_8H_8O
Molecular weight	120.16
Description	Colorless or straw-colored liquid
Vapor pressure	0.3 mm Hg at 20^{o}C
Solubility	3 g/L @ 29^{o}C in water, soluble in alcohol, ether, acetone, heptane, carbon tetrachloride
Conversion factor	4.91 μg/m^3 per ppb at 25^{o}C

III. Major Uses and Sources

Styrene-7,8-oxide (SO) does not occur naturally, although it is produced *in vivo* following exposure to another industrial chemical, styrene (Leibman, 1975; IARC, 1994). SO is manufactured commercially from styrene through chemical synthesis. It may be also be formed when styrene containing polyester resins are treated with peroxides, and SO levels from < 0.003 - 0.14 ppm have been detected in personal air samplers among workers in the reinforced plastics industry (IARC, 1994). SO, a chemically reactive compound, is used in the synthesis of agricultural and biological chemicals, in the treatment of textiles and fibers, and as a diluent in the epoxy-resin industry. It is also used as a laboratory agent in biochemical studies (HSDB, 1995).

IV. Effects of Human Exposures

In humans, as in other animals, SO metabolism is initiated by enzyme-mediated hydration to phenylethylene glycol (styrene glycol). In humans, subsequent metabolism leads primarily to urinary mandelic acid (MA) and phenylglyoxylic acid (PGA) (Bardodej and Bardodejova, 1970; Leibman, 1975; Guillemin and Bauer, 1979). Other pathways that may be present in other animals (in particular conjugation of SO with glutathione via glutathione transferase) are either absent or quantitatively negligible in humans, except when the level of SO is high (Guillemin and Berode, 1988; Chakrabarti *et al.*, 1993; Hallier *et al.*, 1995). The ingestion of ethanol interferes with the formation of MA, but normal MA levels return when ethanol is removed from the diet (Berode *et al.*, 1986). An important consequence of ethanol related decreased levels of urinary MA is the potential underestimation of exposure to SO or the parent solvent styrene (Guillemin and Bauer, 1979; Berode, at al., 1986).

Hydration of SO can take place in the endoplasmic reticulum or cytosolic compartment of the cell (Pacifici *et al.*, 1983; Petruzelli *et al.*, 1992). The presence of a cytosolic hydrase is important for the hydration of environmental SO which does not have to diffuse out of the endoplasmic reticulum as does the biologically formed SO (Oesch, 1972). In human lung, the cytosolic epoxide hydrase is more active than the activity in the endoplasmic reticulum (Petruzzelli *et al.*, 1992), although the cytosolic substrate was *trans*-stilbene oxide, in which a hydrogen of styrene oxide is replaced by a phenyl group. In human adult liver, the activity responsible for the hydration of styrene oxide is present in greater amounts in the endoplasmic reticulum than in the cytosolic fraction (Pacifici *et al.*, 1983). Styrene oxide hydrase in humans is less active than the monkey enzyme and more active than the hydrase(s) in rabbit, rat, or mouse. In experimental animals, SO hydrating activity is greater in liver and kidney than in intestine and lung (Oesch, 1972; Mendrala *et al.*, 1993). This distribution may partially explain the presence of SO in the blood of workers exposed to styrene (Korn *et al.*, 1994). Compared to adult human liver, the cytosolic hydration of SO in fetal human liver appears to take place at a lower rate (28%) (Pacifici *et al.*, 1983). The comparison, however, was not between fetus and mother, but rather between fetal tissue and tissue from adults undergoing abdominal surgery.

Human non-cancer health effects studies specific for SO are not published. Based on the assumption that SO is the styrene metabolite that is responsible for observed human non-cancer health effects, epidemiologic studies suggest but do not prove exposure to SO may lead to disturbances of the central nervous system (CNS) (Mutti, 1993; Rebert and Hall, 1994). While SO probably does play a role in styrene related toxicity, other mechanisms exist including the effect of the metabolites MA + PGA on the dopaminergic system (Mutti, 1993). This postulated mechanism also involves SO, which is required for the pathway that leads to MA + PGA.

V. Effects of Animal Exposures

Rats and rabbits were exposed by inhalation to SO in a study designed to investigate the effect of the chemical on reproductive and developmental function (Sikov *et al.*, 1986). Rats were exposed pregestationally and gestationally and the rabbits were exposed only during the gestational period. The regimen for the rats was 7hr/d, 5d/wk, 3 weeks for pregestational exposure, and 7 hr/d, 7 d/wk, 18 days for gestational exposure. The gestationally exposed rabbits inhaled SO for 7 hr/d, 7d/wk, 24 days.

For both species, the lethality was a major effect. Among rats, deaths occurred at 100 ppm SO, and among the rabbits, dose-dependent increased death rates were observed at 15- and 50 ppm SO. Among the survivors, decreased body weight gain and food consumption were observed following exposure (rats, 100 ppm; rabbits, 15-50 ppm). Among the rats exposed pregestationally or gestationally, increased relative (weight of organ / body weight) lung and kidney weights were observed. Among the gestationally exposed rabbits, increased relative lung weights were recorded. A dose-dependent increase in epithelial hyperplasia was observed in rabbits, although the authors consider the effect as mild. The rabbits also exhibited a dose-dependent increase in bronchitis/bronchiolitis (2/8-0 ppm, 5/8-15 ppm; 4/4-50 ppm). Although the authors suggest this effect may be partially explained by an infection present in the colony, the dose-dependence suggests a chemically-related effect was present.

In the rabbits, statistically significant increased resorptions (postimplantation loss) at 15 and 50 ppm were observed, whereas in the rat, statistically significant decreases in pregnancy occurred at 100 ppm. Decreased corpora lutea were observed in rats exposed pregestationally to 100 ppm SO, but the decrease was not statistically significant. In both species, little or no other feto- or developmental toxicity was observed. The subchronic study described by Sikov *et al.* (1986) is the only reported study that utilized SO inhalation exposure.

Lijinsky (1986) and Conti *et al.* (1988) exposed rodents to SO by ingestion, specifically gavage. In the Lijinsky (1986) study, rats were exposed to 0, 275, 550 (MTD) mg/kg, and mice were exposed to 0, 375, 750 (MTD) mg/kg, 3 times/week for 104 weeks (2-years). High-dose death rates were large, and mean body weights were decreased, compared to controls, although the female rats appeared to be more resistant. Similar results were obtained with mice. SO exposure led to basal cell hyperplasia and/or hyperkeratosis of the forestomach in rats, while in mice, an increased incidence of lipoid degeneration, focal necrosis, and hemorrhage was present at the MTD. In Conti *et al.* (1993), rats were exposed by gastric intubation to 0, 50, and 250 mg/kg, 4-5 days/week, for 52 weeks. According to the narrative, a slight increase in mortality occurred for males but not females (data not given), and no changes in body weights were recorded.

Embryolethality was observed in chick embryos at SO levels $\geq$ 2 μmoles/egg and was more severe at the earlier stages of development (Vainio *et al.*, 1977). Similar results were obtained by Kankaanpa *et al.* (1979) who observed increased lethality and malformations when chick eggs were treated with 0.8 μmole SO. Both endpoints were enhanced in the presence of an inhibitor of SO hydration, although the enhancement was more pronounced for lethality. The result indicates another SO metabolite may play a role in some toxicity endpoints of SO, as has been

suggested by Mutti (1993). When whole rat embryo cultures were exposed to SO, statistically significant decreases in yolk sac diameter, crown-rump length, somite number and embryonic protein content were observed at concentrations equivalent to 0.038 μmoles/ml serum (NOAEL = 0.024 μg/ml serum) (Brown-Woodman *et al.*, 1994). Qualitatively similar results were obtained by Gregotti *et al.* (1994), but the SO levels were expressed as weight SO/culture dish and therefore the doses cannot be compared. In the latter study, dose-dependent embryolethality was observed. The concentration of SO in the culture plate needed for 50 percent lethality was 32 μg/ml, whereas the amount needed for 50 percent malformation (mainly open neural tubes) was 19 μg/ml. Gregotti *et al.* (1994) also studied the effect of SO on embryonic cells from the midbrain and the forelimb buds, wherein differentiation, growth, and viability were evaluated. Although SO affected all parameters, only altered forelimb bud differentiation occurred at SO levels less than that required for forelimb bud growth or viability or midbrain differentiation, growth, or viability. These results suggest SO has the capacity to affect various aspects of embryo growth and development, and isolating one parameter from another may be difficult. The use of the embryo cultures and embryonic cell cultures permits an evaluation of embryonic growth and development in the absence of maternal toxicity.

VI. Derivation of Chronic Reference Exposure Level (REL)

Study	Sikov *et al.*, 1986
Study population	Rabbits
Exposure method	Discontinuous inhalation
Critical effects	Bronchitis, increased mortality, decreased body weight gain, decreased food consumption
LOAEL	15 ppm
NOAEL	Not observed
Exposure continuity	7 hr/d, 7 days/wk
Exposure duration	24 days
Average experimental exposure	4.4 ppm for LOAEL group
Human equivalent concentration	3.8 ppm for LOAEL group (gas with tracheobronchial effects, RGDR = 0.88 based on estimated body weight of 2.98 kg, minute volume of 1.13 L/min, and tracheobronchial surface area of 300 cm^2)
LOAEL uncertainty factor	10
Subchronic uncertainty factor	10
Interspecies uncertainty factor	3
Intraspecies uncertainty factor	10
Cumulative uncertainty factor	3,000
Inhalation reference exposure level	0.001 ppm (1 ppb; 0.006 mg/m^3; 6 $\mu g/m^3$)

Sikov *et al.* (1986) is the only study wherein experimental animals were exposed by inhalation to SO. The study demonstrates that such exposure may lead to maternal effects that result in decreased abilities to carry a pregnancy to term and to protect against infection. In this study, the presence of dose-dependent bronchitis/bronchiolitis may be one factor that led to the dose-dependent lethality for rabbits exposed to SO. Such maternal toxicity may also explain, in part, the absence of observed feto- or developmental toxicities, which are observed in organ and cell culture studies.

Human studies on the effect of exposure to SO were not found. Human studies on the effect(s) of exposure to styrene (the biological precursor to SO), among women in the workforce, are not conclusive, possibly due to low study power due to small numbers of women in the industry under study.

A comparison between the ratio (SO/styrene) for dose-dependent parameters in the embryo- and cell culture studies (Brown-Woodman *et al.*, 1994; Gregotti *et al.*, 1994) and for the chronic RELs is instructive. From the Brown-Woodman *et al.* (1994) study, the SO/styrene = 0.038 for embryotoxicity. In the Gregotti *et al.* (1994) study, which measured 50 percent effective doses(EC_{50}), the EC_{50} for styrene was not attained. Therefore the SO/styrene ranged from <0.022 - <0.077 for differentiation; from <0.065-<0.092 for cytotoxicity; and from <0.15 to <0.26 for cell growth. The SO/styrene = 0.006 for the chronic RELs. The data therefore suggest that the SO doses, present in the Sikov *et al.* (1986) rabbit study, probably affect general biologic endpoints of cell toxicity and growth.

The strengths of the inhalation REL include the availability of controlled exposure inhalation studies in multiple species. Major areas of uncertainty are the lack of adequate human exposure data, the lack of chronic inhalation exposure studies, and the lack of observation of a NOAEL.

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CHRONIC TOXICITY SUMMARY

SULFURIC ACID

(dithionic acid; pyrosulphuric acid)

CAS Registry Number: 7664-93-9

I. Chronic Toxicity Summary

Inhalation reference exposure level	**1 µg/m^3**
Critical effect(s)	Bronchiolar epithelial hyperplasia, and thickening of the bronchial walls in monkeys
Hazard index target(s)	Respiratory system

II. Physical and Chemical Properties (HSDB, 1994)

Molecular formula	H_2SO_4
Molecular weight	98.1 g/mol
Description	Colorless liquid
Specific gravity	1.84 @ 15° C
Boiling point	315-388° C
Vapor pressure	0.001 torr @ 20° C
Solubility	Soluble in water
Conversion factor	Not applicable

III. Major Uses or Sources

Sulfuric acid is a strong acid utilized as an intermediate in the synthesis of linear alkylbenzene sulfonation surfactants used in dyes, in petroleum refining, for the nitration of explosives, in the manufacture of nitrocellulose, in caprolactam manufacturing, and as a drying agent for chlorine and nitric acid.

IV. Effects of Human Exposures

Workers in the lead battery industry showed etching and erosion of the teeth after 4 months exposure to an average concentration of 0.23 mg/m^3 H_2SO_4 (Gamble *et al.*, 1984). Dental erosion increased in a dose-dependent manner with longer durations of exposure.

A study of 33 storage battery plant workers exposed to H_2SO_4 concentrations as high as 35 mg/m^3 showed a greater group mean decrease in FEV_1 across the time of their work shift

compared to workers who were not exposed to sulfuric acid (El-Saddik *et al.*, 1972). The salivary pH of the sulfuric acid exposed workers, a qualitative measure of acid exposure, was lower than the controls during the course of the work shift.

A large number of epidemiologic studies have been conducted showing that elevated levels of several air pollutants, including acid aerosols, sulfur and nitrogen oxides, and particulate sulfates are correlated with an increased prevalence of pulmonary disease (U.S. EPA, 1989). Elevated sulfate levels (1.6 ppb or 6.6 $\mu g/m^3$) have been associated with statistically significant decrements in FVC and FEV_1 in a cohort of Canadian children (Stern *et al.*, 1988). Further analysis of these data led Bates and Sitzo (1989) to conclude that H_2SO_4 was the most likely cause for the pulmonary changes observed. Similarly, Ostro *et al.* (1989) reported a statistical association between asthma-related symptoms reported by 209 asthmatics and sulfate and acidity levels in ambient air in Denver.

Sulfuric acid and oleum are absorbed as salts of sulfate anion (SO_4^{2-}), and are excreted as organic sulfates, neutral sulfur, or neutral sulfur compounds such as sulfur-containing amino acids. The low systemic toxicity of these metabolites is likely of secondary importance to the irritation caused by the inhaled acid.

V. Effects of Animal Exposures

An exposure of 9 monkeys per group to H_2SO_4 concentrations of 0, 0.38, 0.48, 2.43, and 4.79 mg/m^3 continuously for 78 weeks resulted in dose-dependent adverse histological changes in lung and bronchiolar epithelial and parenchymal tissue in addition to a dose-dependent decrease in blood oxygenation (Alarie *et al.*, 1973). In the animals exposed to 0.38 mg/m^3, significant bronchiolar epithelial hyperplasia was observed in 5 of 9 animals; thickening of the bronchiolar walls was observed in 3 of 9 animals. A slight focal bronchial epithelial hyperplasia was present in 4 of the 9 animals. One animal died after 4 weeks exposure to 0.38 mg/m^3. Although signs of pulmonary edema and cardiac hypertrophy were found, the cause of death was not determined.

Alarie *et al.* (1973) also exposed groups of 50 guinea pigs of each sex to 0, 0.08, or 0.1 mg/m^3 continuously for 52 weeks. The group receiving 0.1 mg/m^3 also received larger sized particulates than the 0.08 mg/m^3 group (2.78 μm vs.0.84 μm, respectively). No exposure related effects were observed in the animals exposed to 0.08 mg/m^3 whereas exposure of 0.1 mg/m^3 resulted in decreased body weights in the female guinea pigs. No other histological changes in any organs were observed at the end of the 52-week study.

Rabbits (4 per group) exposed to 250 $\mu g/m^3$ H_2SO_4 1 hour/day, 5 days/week for 4, 8, or 12 months showed significantly increased bronchoconstriction upon acetylcholine challenge after 8 and 12 months exposure compared with a control group of 4 animals that received no H_2SO_4 (Gearhart and Schlesinger, 1986, 1988). Mucociliary clearance was also impaired by H_2SO_4 exposure and did not improve 3 months after cessation of exposure. A decline in dynamic lung

compliance was observed after 12 months exposure. There was no evidence of inflammatory cell infiltration in the lungs of the exposed animals.

In guinea pigs, significantly slower and irregular breathing patterns were noted when the animals had inhaled albumin followed by 30 minute exposures to H_2SO_4 at 1.91 mg/m^3 twice per week for 5 weeks (Kitabatake *et al.*, 1979). Similarly, when guinea pigs were exposed to 2.49 mg H_2SO_4/m^3 for 4 hours/day, 6 days/week for 4 weeks, *in vitro* lung histamine release was significantly enhanced following heterogeneous albumin inhalation compared to control animals unexposed to albumin (Fujisawa *et al.*, 1986; Iguchi *et al.*, 1986). In guinea pigs, sulfuric acid caused significantly greater lung function changes when adsorbed on the surface of zinc oxide particles as compared with pure sulfuric acid (Amdur and Chen, 1989). An exposure to 24 µg/m^3 sulfuric acid, layered on zinc oxide, produced significant reductions in lung function when followed by a brief exposure to 0.15 ppm ozone (Chen *et al.*, 1991).

A chronic exposure of beagle dogs to an average concentration of 889 µg/m^3 for 21 hours/day over a 620 day period resulted in increased expiratory resistance, reduced carbon monoxide diffusing capacity, reduced total and residual lung volume, and decreased lung and heart weights (Lewis *et al.*, 1973).

In apparent contrast to the above studies, rats and guinea pigs exposed to H_2SO_4 at 10 mg/m^3 for 6 hours/day, 5 days/week for 6 months exhibited no adverse histologic changes in lung tissue. Lung function measurements were not reported in this study (Cavender and Singh, 1978).

In mice, inhalation of sulfuric acid mist at a concentration of 1.4 mg/m^3 in combination with a carbon particle mixture (1.5 mg/m^3) for 3 hours/day, 5 days/week for up to 20 weeks, resulted in significant alterations in specific antibody titer (decreased IgG, Ig_{2a}, IgM; increased IgG_{2b}), depression of primary splenic antibody response, and decreased resistance to respiratory infection as measured by mortality and survival time compared to controls (Fenters *et al.*, 1979).

There are no reliable studies indicating that sulfuric acid is a developmental or reproductive toxicant. In the absence of massive overexposure leading to maternal acidemia, H_2SO_4 will be neutralized in the maternal circulation and is unlikely to reach the fetus.

VI. Derivation of Chronic Reference Exposure Level

Study	Alarie *et al.*, 1973
Study population	Cynomolgus monkeys (5 males and 4 females per group or vice versa)
Exposure method	Continuous inhalation exposures (0, 380, 480, 2400, or 4800 µg/m^3) for 78 weeks
Critical effects	Significantly increased bronchial epithelial hyperplasia and bronchial thickening
LOAEL	380 µg/m^3
NOAEL	Not observed

Exposure continuity	The exposure was continuous during the experiment
Exposure duration	78 weeks
Average experimental exposure	380 μg/m^3 for the LOAEL group
Human equivalent concentration	130 μg/m^3 (RGDR assumed to equal 0.33)
LOAEL uncertainty factor	3 (slight, low incidence effects)
Subchronic uncertainty factor	1 (see text)
Interspecies uncertainty factor	3
Intraspecies uncertainty factor	10
Cumulative uncertainty factor	90
Reference exposure level	1 μg/m^3

The study by Alarie *et al.* (1973) identified a LOAEL for chronic exposure to sulfuric acid of 380 μg/m^3. The principal uncertainties of this study are the small sample size of the test groups and the absence of an observed NOAEL. A lower chronic LOAEL for bronchial reactivity is presented by Gearhart and Schlesinger (1986, 1988) for rabbits (250 μg/m^3). The reason this study was not selected as the basis of the REL was that, in their study, Gearhart and Schlesinger used only a single concentration of sulfuric acid, exposed the animals only for 1 hour per day for 5 days/week, used only 4 animals per group, and measured effects over the course of up to 12 months. The predominant weakness in the rabbit study however, was the extreme discontinuity of the exposures (1 hour/day, 5 days/week) which would have necessitated a very large continuity adjustment assumption. For these reasons, in addition to obvious physiological and genetic similarity arguments, the study in monkeys by Alarie *et al.* (1973) was felt to be more appropriate as the basis for the chronic REL for sulfuric acid.

A free-standing NOAEL for histological changes in 100 guinea pigs exposed continuously for 1 year to 0.08 mg/m^3 was reported by Alarie *et al.* (1973). Guinea pigs respond to high concentrations of sulfuric acid by occasional laryngeal spasms that appear similar to a human asthmatic attack (Silbaugh *et al.*, 1981; Amdur 1989). As a result, guinea pigs are thought to be sensitive models for the acute effects of sulfuric acid. For chronic effects of sulfuric acid on the lung, monkeys are likely a suitable model due to physiological structural similarites to humans. Although the duration of exposure in the monkey study was 1.5 years (less than 10% of an average monkey's lifespan of 35-40 years; U.S. EPA, 1988) and therefore technically subchronic, no histological effects were observed in guinea pigs exposed chronically (i.e. 1 year out of a 5 year lifespan) to a concentration one-fifth that of the LOAEL in monkeys. Therefore, an uncertainty factor for subchronic to chronic exposures appears to be unnecessary.

For comparison, an REL based on the guinea pig NOAEL of 0.08 mg/m^3 would be 0.8 μg/m^3.

The major strengths of the study is the use of health effects observations from continuous long-term exposures to a primate. The major weaknesses are the lack of adequate human health effects data and the lack of a NOAEL observation.

The occupational standard for sulfuric acid is based on a study in human subjects by Amdur *et al*. (1952). In their study, 22 healthy male subjects were exposed to 0, 0.35, to 5 mg/m^3 for 5-15 minutes. The odor, taste, and irritation threshold was 1 mg/m^3. Since the basis for this standard is an acute exposure, it is not useful in determining a chronic non-cancer REL for sulfuric acid. A review of chronic human exposures to sulfuric acid and resulting carcinogenicity outcomes cane be found in IARC (1992). However, none of the studies in that review examined non-cancer endpoints.

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CHRONIC TOXICITY SUMMARY

2,3,4,6-TETRACHLOROPHENOL

(Synonym: Dowicide 6)

CAS Registry Number: 58-90-2

I. Chronic Toxicity Summary

Inhalation reference exposure level	**90 μg/m^3**
Oral reference exposure level	**0.03 mg/kg-day**
Critical effects(s)	Liver centrilobular hypertrophy in rats
Hazard index target(s)	Alimentary system

II. Chemical Property Summary (HSDB, 1995)

Molecular formula	$C_6H_2C_{14}O$
Molecular weight	231.89
Description	Solid (needles from ligroin or acetic acid, brown flakes or sublimed mass)
Vapor pressure	1 mm Hg @ 100^{o}C
Solubility	0.01 g/100 ml water @ 25^{o}C, soluble in acetone, hot acetic acid, methanol, benzene, chloroform, sodium hydroxide
Conversion factor	9.48 μg/m^3 per ppb at 25^{o}C

III. Major Uses and Sources

Tetrachlorophenol (TeCP) is prepared industrially by the chlorination of phenol under varying conditions of temperatures, solvents and catalysts (IARC, 1986). TeCP is used as an intermediate in the chemical synthesis of herbicidal chlorophenoxy acids, and is often a contaminant of pentachlorophenol preparations (HSDB, 1995). The sodium salt of TeCP has been used as a fungicide (IARC, 1986). During wood treatment processes, TeCP is a major component of the aqueous dipping baths that contain chlorophenate salts, and in some commercial wood treatment chlorophenolate formulations, TeCP is present in larger amounts than pentachlorophenol (Kalman and Horstman, 1983; Embree *et al.*, 1984; Valo, 1984; Pekari *et al.*, 1991).

IV. Effects of Human Exposures

Kalman and Horstman (1983) studied the urinary elimination of TeCP in 40 factory woodworkers exposed to a wood preservation agent that contained 21 percent TeCP (air levels < 0.52 mg/m^3 TeCP). Urinary levels in exposed workers (>100 ppb) decreased by 84 - 90 percent during a 1-2 week vacation interval, whereas the levels in low- or unexposed workers (<20 ppb) decreased by only 34 percent. The decreased reduction among the low- or unexposed workers may be related to the background levels in the population. Among 7 sawmill workers, exposed to a wood preservative containing 74 percent TeCP, about 80 percent of the urinary TeCP was present as the sulfate conjugate (Pekari *et al.*, 1991).

Serum TeCP values of 1-6 μM were reported among 7 sawmill workers who were continuously to a product containing 74 percent TeCP (Pekari *et al.*, 1991). Among these workers, no changes in TeCP serum levels occurred between the start of work in the morning and the end of the shift in the afternoon.

Urinary elimination half-lives ($T_{1/2}$) were calculated from excretion data obtained from wood workers first exposed to a wood preservation agent during normal work routine and then followed after the use of the agent was stopped. Using a one compartment model, a $T_{1/2}$ of 4.8 days (Kalman and Horstman, 1983) or 4.2 days (Pekari *et al.*, 1991) was calculated. Pekari *et al.* (1991) state that the their data are better explained by a two compartment model, and they calculate a $T_{1/2}$ (distribution) of 4.2 days and a $T_{1/2}$ (elimination) of 26 days. Pekari *et al.* (1991) also showed that the workers continued to excrete TeCP when they were working in the plant during the interval when the TeCP preservation agent (74 percent TeCP) was not in use. Although such data could represent a slow release of TeCP stored in the tissues, it may also have been due to the residual TeCP in the environment of the factory (Valo *et al.*, 1984).

Similar to results obtained from *in vitro* studies in experimental animals, TeCP is active in human cell systems. Janik and Wolf (1992) report the inhibition of the human erythrocyte Ca^{2+} - transport - ATPase system by a series of chlorinated phenols, including TeCP. In this system, the amount of TeCP required for 50 percent inhibition is 150 μM, compared to 1000 - 1500 μM for dichlorophenols, 300 μM for 2,3,4-trichlorophenol, and 20 μM for pentachlorophenol.

V. Effects of Animal Exposures

In rats, 2,3,4,6-TeCP, administered by intraperitoneal injection (ip) (total dose of 4.9 mg), is excreted as the parent compound (96 percent) and a minor amount as trichloro-*p*-hydroquinone, (Ahlborg and Larsson, 1978). Qualitatively similar results were obtained with 2,3,4,5-TeCP (total dose of 4.9 mg), although in smaller yields. Exposure, by ip injection, of the rats to 2,3,5,6-TeCP (total dose of 5.3 mg), results in the urinary excretion of 65 percent unmetabolized compound and 35 percent tetrachloro-*p*-hydroquinone. These results are consistent with the rat metabolite profile of pentachlorophenol, wherein the three TeCP isomers and tetrachloro-*p*-hydroquinone were identified following oral administration, although the corresponding catechol and resorcinol were also observed (Renner and Hopfer, 1990). The role of oral vs. ip

administration exposure was not investigated, although Ahlborg and Larsson (1978) demonstrate enhanced acute toxicities of the parent tetrachlorophenols and some metabolites following ip injection compared to oral exposure. Because the urinary extracts were acid hydrolyzed for analysis, no information on the extent of urinary conjugation was obtained.

In the presence of 2,3,4,6-TeCP, the metabolism of tertiary aromatic amines shifts from cytochrome P450-mediated C-oxygenation to flavin-mediated N-oxygenation (Arrhenius *et al.*, 1977). N-oxygenation of tertiary aromatic amines may be associated with detoxiciation or bioactivation (Damani, 1983). Hence, such a shift in metabolism could have toxicologic consequences for multiple exposures that contain TeCP.

Other TeCPs, i.e. 2,3,5,6- and 2,3,4,5- TeCP interfere with the vitamin K-dependent γ-carboxylation of protein glutamic acid residues, however, the 2,3,4,5-isomer is ten to twenty less active as an inhibitor (Grossman and Suttie, 1990. The 2,3,4,6-TeCP was not tested in this *in vitro* system, and pentachlorophenol behaved similarly to the 2,3,4,5-TeCP. The concern about interference with the γ-carboxylation of glutamate residues results from the requirement for this reaction in the blood clotting mechanism.

In a U.S. EPA sponsored study (American Biogenics, 1986),30 female (F) and 30 male (M) rats were exposed by oral gavage to TeCP (0, 25, 100, 200 mg/kg-day for 91-93 days) and signs of toxicity were recorded (U.S. EPA, 1986). No treatment related deaths occurred. A NOAEL of 25 mg/kg-day was determined for clinical and pathologic responses. These responses included decreased blood urea nitrogen (F), increased serum succinate pyruvate transaminase levels (M), increased total protein and albumin levels (M), salivation (F,M), increased absolute and relative liver and kidney weights (F,M), and centrilobular hypertrophy of the liver (M>F). Liver centrilobular hypertrophy was chosen as the end-point for the U.S. EPA reference level (IRIS, 1995).

Rats exposed intragastrically daily for 55 days to 0, 10, 50, or 100 mg/kg 2,3,4,6-TeCP (> 99 percent pure), exhibited residues in the tissues in a dose dependent manner (Hattula *et al.*, 1981). At 50 and 100 mg/kg, the highest concentrations were found in spleen and kidney. The lowest amount was in muscle and the levels in liver and brain were intermediate. According to the text, major tissue damage was to the liver (2/10 with medium to severe changes at 100 mg/kg; 1/10 with severe necrosis at 50 mg/kg; and 0/10 at 10 mg/kg. No statistical analysis was reported. The lack of detail prevents an adequate evaluation of the data for a determination of a NOAEL.

In a study designed to evaluate feto- and developmental toxicity of TeCP, pregnant rats were exposed by gavage to 0, 10, or 30 mg/kg-day of commercial or purified 2,3,4,6-TeCP on gestation days 6 through 15 (Schwetz *et al.*, 1974). The purified TeCP was 99.6 percent pure, whereas the commercial sample was only 73 percent pure with contamination by pentachlorophenol and various polychlorodibenzo-*p*-dioxins and polychlorodibenzfurans. Fetal toxicity (defined as resorptions, decreased body weight, and crown-rump length) was not observed among the pups exposed in utero to the commercial or purified TeCP, and maternal toxicity was not observed among the exposed dams. Subcutaneous edema was observed among the pups exposed to 10 mg/kg-day, but not among those exposed to 30 mg/kg-day. The lack of a

clear dose-response relationship and a short exposure interval (10 days) prevents a quantitative consideration of fetotoxicity.

In a similar study designed to distinguish between maternal and embryotoxicity, pregnant rats were exposed by ingestion to 0, 25, 100, and 200 mg/kg-day TeCP on gestation days 6-15 (U.S. EPA, 1988). Dams were killed on gestation day 20 and examined for toxic responses. Decreased weight gain was observed for dams exposed to 200 mg/kg-day. Like the Schwetz *et al.* (1974) study, the exposure duration was short and not applicable to a chronic toxicity study, although maternal toxicity is suggested.

VI. Derivation of Chronic Reference Exposure Level (REL)

Study	American Biogenics, 1986 (sponsored by U.S. EPA, 1986)
Study populations	Albino rats (30 male and 30 female per dose group)
Exposure method	Oral gavage (0, 25, 100, or 200 mg / kg body weight, in olive oil)
Critical effects	Liver centrilobular hypertrophy
LOAEL	100 mg/kg-day
NOAEL	25 mg/kg-day
Exposure continuity	Discontinuous daily gavage exposure
Exposure duration	90 days
Average experimental exposure	25 mg/kg body weight per day
LOAEL uncertainty factor	1
Subchronic factor	10
Interspecies uncertainty factor	10
Intraspecies uncertainty factor	10
Cumulative uncertainty factor	1000
Oral reference exposure level	0.03 mg/kg-day (U.S. EPA RfD)
Route-to-route extrapolation factor	3,500 $\mu g/m^3$ per mg/kg-day
Inhalation reference exposure level	0.09 mg/m^3 (90 $\mu g/m^3$; 0.009 ppm; 9 ppb)

The American Biogenics (1986) study represents the only available detailed toxicologic analysis of 2,3,4,6-TeCP. Thirty rats per sex and three dose levels were used. The purity of the test agent was monitored and statistical analyses were applied to the observations. A major difficulty with the use of this study is the absence of the original data from the report. Hence the NOAEL / LOAEL must be determined from the narrative, which is detailed.

Hattula *et al.* (1981) also observed liver damage in rats exposed intragastrically to purified TeCP for 55 days. According the text, a NOAEL of 10 mg/kg-day was indicated. However, statistical analysis was not carried out and few details were available for evaluation. For these reasons, the U.S. EPA (1986) study was considered more appropriate for the development of an REL.

Human data on the toxicologic effect(s) of TeCP were not found. Human data would be expected to be difficult to evaluate in terms of TeCP itself, because human exposures typically include TeCP in mixtures that contain other known toxicants, such as pentachlorophenol, polychloro-p-dibenzodioxins, and polychlorodibenzfurans.

A major uncertainty in the inhalation REL for TeCP is the use of a direct route-to-route extrapolation. No inhalation data on the effects of TeCP were found in the literature, and the inhalation REL is therefore based on an ingestion study with the use of the default values for average human weight (70 kg) and a daily inhalation rate (20 m^3/day). Tetrachlorophenols are readily absorbed from the gastrointestinal tract, from parenteral sites of injection, from skin, and from the respiratory tract (HSDB, 1995). This information together with a log Kow = 4.1 is considered supportive of the decision to use a direct route-to-route extrapolation for the 2,3,4,6-inhalation REL in the absence of adequate inhalation data.

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CHRONIC TOXICITY SUMMARY

TOLUENE

(Methyl benzene; methyl benzol; phenyl methane; toluol)

CAS Registry Number: 108-88-3

I. Chronic Toxicity Summary

Inhalation reference exposure level	**400 µg/m^3** (U.S. EPA RfC) This document summarizes the evaluation of non-cancer health effects by U.S. EPA for the RfC
Critical effect(s)	Neurological disturbances in human workers
Hazard index target(s)	Nervous system; alimentary system; teratogenicity

II. Physical and Chemical Properties (HSDB, 1995 except as noted)

Description	Colorless liquid
Molecular formula	C_7H_8
Molecularweight	92.13 g/mol
Specific gravity	0.861 @ 25°C (Low *et al.*, 1988)
Boiling point	111° C
Vapor pressure	28.1 mm Hg @ 25°C (U.S. EPA, 1984)
Solubility	miscible in most organic solvents
Conversion factor	1 ppm = 3.77 mg/m^3 @ 25°C

III. Major Uses or Sources

Toluene occurs naturally as a component of crude oil and is produced in petroleum refining and coke oven operations (HSDB, 1995). It is used in household aerosols, nail polish, paints and paint thinners, lacquers, rust inhibitor, adhesives and solvent based cleaning agents. Toluene is also utilized in printing operations, leather tanning and chemical processes. Benzene and other polycyclic aromatic hydrocarbons are common contaminants of toluene. Toluene is considered a sentinel chemical for benzene in the context of air and water sample monitoring.

IV. Effects of Human Exposures

Case studies of solvent abusers have shown that high doses of toluene (e.g. 425 mg/day) can cause neurobehavioral changes and degeneration of cerebellar, coritcal, and brainstem functions.

A battery of neurobehavioral tests was performed in 30 female workers exposed to toluene vapors in an electronic assembly plant (Foo *et al.*, 1990). The average number of years worked was 5.7 ± 3.2 for the exposed group and 2.5 ± 2.7 years for the controls. The exposed group of workers inhaled a time-weighted average of 88 ppm (330 mg/m^3) toluene while the control workers inhaled 13 ppm (49 mg/m^3). A significant decrease in neurobehavioral performance was observed in the exposed workers in 6 out of 8 tests. Irritant effects were not examined, and concurrent exposures to other chemicals was not addressed. In this study, 88 ppm was considered a LOAEL for central nervous system effects.

Workers exposed to lesser concentrations have shown some impairment of CNS endpoints, however, controls and exposed individuals have not been well matched in many of these studies (Hanninen *et al.*, 1987; Iregren, 1982; Cherry *et al.*, 1985).

Solvent workers were exposed to 42.8 ppm toluene (estimated as a time-weighted average) for an average duration of 6.8 years (Yin *et al.*, 1987). No significant differences from controls were noted in treated individuals in questionnaires, hematology, or urinalyses. This study did not account for confounding variables, such as smoking and alcohol consumption.

Subjective symptoms of headache, sore throat and dizziness were reported by workers exposed to approximately 100 ppm toluene (time-weighted average, duration unspecified) (Lee *et al.*, 1988). The prevalence of these symptoms was concentration-dependent. A similar study examined the psychomotor, manual dexterity, and visual perception abilities of college students exposed to 0, 74, or 151 ppm (0, 278, or 566 mg/m^3) toluene 7 hours/day for 3 days (Echeverria *et al.*, 1989). In addition to the above objective measures, subjective symptoms of eye irritation, headache, and somnolescence were noted at 151 ppm. Visual perception and manual dexterity performances both decreased, while reported symptoms increased in the 151 ppm group. No effects of toluene on psychomotor tests were observed. In this study, 74 ppm was a NOAEL

Baelum *et al.* (1985) found that subjects exposed to 0 or 100 ppm (375 mg/m^3) toluene for 6.5 hours experienced a loss of color discrimination, regardless of their prior solvent exposure history. Other signs of toxicity included visual perception and visual motor function, although these signs were only observed in the occupationally-exposed individuals.

Liver toxicity has been documented in toluene solvent abusers (Fornazzari *et al.*, 1983) and in a cross-sectional study of 289 printing workers exposed to an estimated 53 ppm (200 mg/m^3) for 8 hours/day, 8 workers had significantly elevated serum enzymes (ALT/AST ratio) indicative of liver damage (Guzelian *et al.*, 1988). However, another cross-sectional study by Boewer *et al.* (1988) showed no significant effects on serum enzymes in 181 printing workers exposed to concentrations below 53 ppm (200 mg/m^3).

Toluene was identified as the principal solvent associated with an increased incidence of urinary tract birth defects in a retrospective cohort study of 301 cases compared with 301 referent controls (McDonald *et al.*, 1987). The cohort was matched for age, employment, date of delivery and educational level.

V. Effects of Animal Exposures

Rats (20 per group) exposed for 2 years to 0, 600, or 1200 ppm (0, 2261, or 4523 mg/m^3) toluene 6.5 hours/day, 5 days/week for 103 weeks were examined for hematological and histopathological effects in addition to gross observations of toxicity (NTP, 1990). Significant erosion of the olfactory epithelium was observed in male rats while degeneration of the respiratory and nasal epthelium was observed in both sexes at 600 ppm.

A study of the chronic effects of toluene in rats (5-20 animals per group) exposed for 106 weeks to 0, 30, 100, or 300 ppm (0, 113, 375, or 1125 mg/m^3) showed no treatment-related effects on histopathology of major organs, including the nasal turbinates (CIIT, 1980). In this study, the samples taken for nasal histopathology examination may have been inadequate to substantiate the nasal lesions reported by the NTP (1990).

Reproductive toxicity to maternal rats was observed during exposure to 1500 ppm toluene, 24 hours/day on days 9 to 14 of gestation (Hudak and Ungvary, 1978). In this experiment, 2 dams out of 19 died during exposure. Fetuses from the 1500 ppm group showed increased incidence of sternebral alterations, extra ribs and missing tails. The same concentration given on days 1 through 8 of gestation resulted in 5 deaths out of 14 dams. Fetuses in this regimen showed increased incidence of hydrocephaly and growth retardation compared to controls. A third regimen that exposed maternal rats to 1000 ppm on days 1 through 21 of gestation resulted in no maternal deaths or toxicity, and an increase in the incidence of skeletal variations in the fetuses. When exposed to 1500 ppm continuously, maternal mice died within 24 hours of exposure whereas exposure to 500 ppm had apparently no effect. Examination of the fetal mice showed significant growth retardation in the 500 ppm group.

Inhalation of 0 or 800 mg/m^3 toluene for 6 hours/day on gestation days 14-20 (rats), or days 6-11 (hamsters) showed significant exposure-related decrease in birth weight of the rats compared with controls (Da Silva *et al.*, 1990). In addition to low birth weight, the numbers of live pups was significantly lower in the 800 ppm group. No deficits in any parameter were noted in the hamsters. In this study, no neurobehavioral effects were noted in the offspring.

A 2-generation study of the effects of 0, 100, 500, or 2000 ppm (0, 377, 1885, or 7538 mg/m^3) toluene in rats (males, 10-40 per group; females, 20-80 per group) (API, 1985). Rats were exposed for 6 hours/day, 7 days/week for 80 days and a 15 day mating period. The mated females were then exposed to the same concentrations during days 1-20 of gestation and days 520 of lactation. Afler weaning, the F1 pups were exposed 80 times to the appropriate exposure group and then randomly mated to members ofthe same exposure group. The F1 generation showed significantly decreased body weight which remained throughout lactation. No effects were observed on histopathology. No data was presented for the F2 generation.

Mice exposed chronically to 0, 120, 600, or 1200 ppm (0, 452, 2261, or 4523 mg/m^3) toluene 6.5 hours/day, 5 days/week, for 2 years (NTP, 1990). The only treatment-related effect was a significant increase in the number of animals with hyperplasia of the bronchial epithelium in the 1200 ppm exposure group.

No significant effects of 1481 ppm toluene exposure were noted in rats (15/sex/group) after 26 weeks exposure(API, 1981). Examined in this study were neurohistopathological responses, hematology, serum enzymes and urinalyses.

Ototoxicity in the form of hearing loss was observed in rats exposed to 1000 ppm 14 hours/day for 2 weeks (Pryor *et al.*, 1984). In this study, the auditory brainstem reponse and behavioral changes both indicated hearing loss. A lower concentration of 700 ppm for 14 hours/day for 16 weeks did not result in any significant hearing loss.

VI. Derivation of U.S. EPA RfC

Study	Foo *et al.*, 1990; NTP, 1990; U.S. EPA, 1994
Study population	30 Female workers in an electronic assembly plant
Exposure method	Occupational inhalation
Critical effects	Neurobehavioral deficits in 6 out of 8 tests
LOAEL	88 ppm
NOAEL	Not observed
Exposure continuity	10 m^3/day occupational inhalation rate, 5 days/week
Average occupational exposure	31.4 ppm
Exposure duration	5.7 $\pm$ 3.2 years (exposed group); 2.5 $\pm$ 2.7 years (controls)
LOAEL uncertainty factor	10
Subchronic uncertainty factor	1
Interspecies uncertainty factor	1
Intraspecies uncertainty factor	10
Modifying factor	3 (database deficiencies including the lack of animal neurotoxicity and irritation studies)
Cumulative uncertainty factor	300
Inhalation reference exposure level	0.1 ppm (100 ppb; 0.4 mg/m^3; 400 $\mu g/m^3$)

The major strength of the U.S. EPA RfC is the use of human exposure data from workers exposed over a period of years. The major weaknesses are the uncertainty in estimating exposure, the potential variability in exposure concentration, and the lack of a NOAEL observation and of dose-response information.

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CHRONIC TOXICITY SUMMARY

2,4- AND 2,6-TOLUENE DIISOCYANATE

(2,4- and 2,6-TDI; 2,4- and 2,6-diisocyanato-1-methylbenzene; 2,4- and 2,6-diisocyanatoluene)

CAS Registry Number: 584-84-9 or 26471-62-5 (mixture)

I. Chronic Toxicity Summary

Inhalation reference exposure level	**0.07 µg/m^3** (U.S. EPA RfC) This document summarizes the evaluation of non-cancer effects by U.S. EPA for the RfC
Critical effect(s)	Decreased lung function in occupationally exposed workers
Hazard index target(s)	Respiratory system

II. Chemical Property Summary (HSDB, 1995)

Molecular formula	$C_9H_6N_2O_2$
Molecular weight	174.15 g/mol
Description	Colorless to pale yellow liquid
Vapor pressure	0.01 mm Hg @ 20°C
Solubility	Miscible with ether, acetone, benzene, carbon tetrachloride, chlorobenzene, diglycol monomethyl ether, kerosene, olive oil, alcohol; soluble in ethyl acetate
Conversion factor	7.1 µg/m^3 per ppb at 25ºC

III. Major Uses and Sources

Commercial toluene is comprised of approximately 80% 2,4-TDI and 20% 2,6-TDI. TDI is used in the manufacture of polyurethane foams, elastomers, and coatings (HSDB, 1995; Howard, 1989). It is also used in the manufacture of floor and wood finishes, lacquers, foam plastics, polyurethane foam coated fabrics, and insulation materials (HSDB, 1995; Howard, 1989; Duncan *et al.*, 1962). Emissions of TDI to the atmosphere can occur during production, handling, and processing of polyurethane foam (Howard, 1989).

IV. Effects of Human Exposures

Diem *et al.* (1982) conducted a prospective study beginning in 1973 of 277 male workers involved in the production of TDI. The study examined pulmonary function, with nine examinations conducted over a five year period. A large group of workers (168) with no previously reported TDI exposure was examined 6 months prior to TDI production in the plant to provide baseline pulmonary function measurements. Personal sampling by continuous tape monitors provided exposure levels, but were not used until 2 years after the study was initiated. Sampling information resulted in a division of the workers into two groups: those exposed to levels below 68.2 ppb-months (which reflects the level of exposure of a worker for the entire 5 year duration in the low-exposure area (geometric mean - 1.1 ppb)) and those above this level. The arithmetic mean exposure level for the non-smokers was 1.9 ppb TDI in the high-exposure group and 0.9 ppb TDI in the low-exposure group (calculated by Hughes, 1993). The higher exposure group was further limited to those individuals who showed a normal FEV_1 to height ratio. Data were analyzed by the maximum likelihood weighted regression approach (Diem and Liukkonen, 1988). Both FEV_1 and forced expiratory flow (25-75%) among workers who never smoked were found to be significantly reduced in the high-exposure group (n=21) compared to the low-exposure group (n=35). Categorizing workers based on time spent at exposure levels above 20 ppb also demonstrated a significant difference in FEV_1 and FEV(25-75%) and this effect was also observed among current smokers. Among low-exposure workers, a smoking effect was observed, with smokers showing a significant decline in FEV_1.

A similar longitudinal study of lung function was conducted among workers exposed to TDI during the course of polyurethane foam production (Jones *et al.*, 1992). Participants (181 males and 46 females) were required to have 3 or more spirometric examinations over the 5 year study period. Exposure of males was evaluated by personal monitors and resulted in arithmetic mean low exposure levels of 0.3, 0.4, and 0.4 ppb TDI for never-smokers, ex-smokers, and current smokers, respectively. Among workers with high-level exposure, mean TDI levels were reported to be 1.3, 1.2, and 1.2 ppb for never-, ex-, and current smokers, respectively. Stepwise multiple linear regression methods (excluding asthmatics) were used in evaluating the data (Diem and Liukkonen, 1988). No relationship between TDI exposure and change in lung function was observed, although the prevalence of chronic bronchitis was significantly associated with exposure.

A longitudinal study of 780 workers exposed to TDI in the production of polyurethane foam was also conducted (Bugler *et al.*, 1991; unpublished). Exposure levels were established using continuous-tape personal monitoring devices. The mean exposure level was 1.2 ± 1.1 (s.d.) ppb TDI among 521 workers and 0.3 ± 0.18 (s.d.) ppb TDI in the control group. Another control group who handled cold urethane products had an 8 hour time-weighted average exposure of 0.6 ppb TDI. No significant longitudinal changes in FEV_1 were found after regression analysis, although FEV_1 decline was high among the control group and exposure levels among the different groups were close, limiting the power of the study to detect changes. Approximately 3% of the 780 workers showed signs of TDI sensitization and, of these, over 80% were in the exposed group.

Meta-analysis of the three data sets (Jones *et al.*, 1992; Bugler *et al.*, 1991; Diem *et al.*, 1982) showed that the difference in significance among the findings of each of the studies could have been due to chance, with the change in the probability density for the decline in FEV_1 shifting in the same direction for all data sets and the smoker/non-smoker slope difference becoming less meaningful with the data set combination (Hasselblad, 1993).

Another toxicological area of concern with exposure to TDI is the development of sensitization, resulting in a well-documented condition known as "isocyanate asthma" of either immediate or delayed-type onset (Moscato *et al.*, 1991). The level of exposure required to either develop or trigger a sensitization reaction is not well documented, however. Weaknesses of studies showing pulmonary effects of TDI exposure include use of area sampling vs. breathing-zone measurement of exposure, poor statement of criteria for evaluating hypersensitivity, and the presence of other compounds in the environment which may influence lung function.

V. Effects of Animal Exposures

Mice were exposed to TDI concentrations ranging from 0.007 to 1.18 ppm for 3 hours/day for 5 days consecutively (Sangha and Alarie, 1979); decreased respiratory rate was observed in groups exposed to levels higher than 0.023 ppm TDI. Groups of four mice were also exposed to 0.031 and 0.250 ppm TDI for 3 hours/day for 3 days. Lesions of the external nares and respiratory epithelium were observed in the high dose group.

Female guinea pigs exposed to 0.12, 0.36, 0.61, 0.96, and 10.00 ppm TDI (head-only) for 3 hours/day for 5 consecutive days (short protocol) or to 0.02 ppm TDI (whole body) plus controls for 6 hours/day, 5 days/week for 70 days (long protocol) showed decreased respiration rate two hours into exposure at levels above 0.12 ppm TDI and cytophilic antibody response at 0.96 ppm and above (Karol, 1983). All animals exposed to 10 ppm died. Dermal sensitivity was evident among animals in the short protocol down to 0.12 ppm TDI. No antibody response or dermal sensitivity developed in the animals exposed to 0.02 ppm TDI in the long protocol, although antibody titer was high.

Similarly, guinea pigs (8 females) were exposed to 1.40 ppm TDI for 3 hours/day for 4 days (head only; no control group). In a second exposure regimen, animals (n=24) were exposed to 0.02 ppm TDI for 6 hours/day, 4 days/week for 70 days (whole body) including a control group (n=8) exposed to room air in a similar manner (Wong *et al.*, 1985). Half the animals (4/8) exposed to 1.40 ppm TDI showed pulmonary hypersensitivity (measured on days 37 and 38) and all developed TDI-specific IgE antibodies, whereas none of the animals in the 0.02 ppm TDI group showed either of these effects. Histopathological effects in the 1.40 ppm TDI group included interstitial inflammation, pleural thickening, and peripheral lymphoid hyperplasia. Interstitial inflammation was noted in 2/24 animals exposed to 0.02 ppm TDI.

SD rats and CD-1 mice were exposed to 0.05 or 0.15 ppm TDI for 6 hours/day, 5 days/week for 2 years (Loeser, 1983; nasal histopathology reported by Owen, 1984). Among female rats at both dose levels and male rats at the high dose level, histopathological effects including necrotic

rhinitis, metaplasia, and inflammation of the respiratory epithelium were observed. Female animals showed dose-dependent increases in incidence and severity of this effect. Similar lesions were reported in mice, although not well-characterized.

Reproductive toxicity of TDI was evaluated in a two-generation study conducted in rats (Tyl and Neeper-Bradley, 1989). Weanling rats (28/sex/dose) were exposed to 0, 0.020, 0.079, and 0.290 ppm TDI for 6 hours/day, 5 days/week, for 10 weeks, at which time the animals were randomly mated. Exposure of the females continued through gestation (excepting gestational day 20 through the fourth day postpartum), and exposure of the males continued only until the delivery of the F_1 generation. Weanlings in the F_1 generation were exposed in a manner similar to the parental (P_0) generation and bred after weaning to produce the F_2 generation. Body weights were significantly reduced among animals of both sexes in the highest dose group and weight gain was reduced among males in the highest dose group. Effects on the respiratory system in the P_0 generation animals included rhinitis of the epithelium in the two highest dose groups of both male and female animals. Hyperplasia of the respiratory epithelium was also increased in the high dose groups of both sexes among P_0 animals. Among males in the F_1 generation, the incidence of rhinitis was significantly increased at all exposure levels and the incidence of submucosal lymphoid infiltrates of the larynx and trachea was increased in the highest dose group. F_2 generation animals showed reduced pup weight and weight gain during the lactation period in the two highest dose groups.

Developmental toxicity of TDI was evaluated by exposing pregnant Sprague-Dawley rats (25/group) for 6 hours/day on gestational days 6-15 to 0, 0.021, 0.120, or 0.48 ppm TDI (Tyl, 1988). Reduced maternal body weight, decreased food consumption, and rales occurred among the dams in the 0.48 ppm TDI dose group. A significant fetal effect, a statistically significant increase in a specific skeletal malformation, was reported in the highest dose group.

VI. Derivation of the U.S. EPA RfC

Study	Diem *et al.*, 1982
Study population	Human TDI production workers (168)
Exposure method	Occupational inhalation exposure
Critical effects	Decreased lung function
LOAEL	0.014 mg/m^3
NOAEL	0.006 mg/m^3
Exposure continuity	8 hrs/day (10 m^3/day occupational exposure), 5 days/week
Exposure duration	5 years
Average occupational exposure	0.002 mg/m^3 for NOAEL group
Human equivalent concentration	0.002 mg/m^3 for NOAEL group
LOAEL uncertainty factor	1
Subchronic uncertainty factor	3
Interspecies uncertainty factor	1
Intraspecies uncertainty factor	10

Cumulative uncertainty factor	30
Inhalation reference exposure level	0.00007 mg/m^3 (0.07 µg/m^3; 0.00001 ppm; 0.01 ppb)

The U.S. EPA selected the human occupational study examining pulmonary effects of TDI for the derivation of the RfC (U.S. EPA, 1995; Diem *et al.*, 1982). The rationale for selection of this study has been adopted for the establishment of the chronic REL. This study presented evidence of a decline in lung function, as indicated by decrements in FEV_1, among workers involved in TDI production. In its evaluation of the study (Diem *et al.*, 1982), U.S. EPA cited factors supporting its quality including: (1) the absence of other confounding compounds in the work environment, (2) establishment of baseline lung function prior to exposure to TDI, (3) "parallel internal comparison" of study groups for lung function, (4) appropriate statistical analysis which took into account interindividual variability, (5) breathing zone measurement of TDI (although commenced 2 years into the study), and (6) a smoking effect on lung function.

The major strengths of the U.S. EPA RfC are the use of human exposure data from workers exposed over a period of years and the observation of a NOAEL. The major weaknesses are the uncertainty in estimating exposure, the potential variability in exposure concentration, and the limited nature of the study which focused on lung effects.

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CHRONIC TOXICITY SUMMARY

1,1,2-TRICHLORO-1,2,2-TRIFLUOROETHANE

(1,2,2-trichlorotrifluoroethane; Freon™ 113; Fluorocarbon 113; FC 113; CFC 113)

CAS Registry Number: 76-13-1

I. Chronic Toxicity Summary

Inhalation reference exposure level	**90,000 μg/m^3**
Critical effect(s)	Decreased body weight gain in rats.
Hazard index target(s)	Alimentary system

II. Chemical Property Summary (HSDB, 1995)

Molecular formula:	$C_2Cl_3F_3$
Molecular weight:	187.38 g/mol
Description:	Colorless liquid which exists as a gas above 47.6°C; nearly odorless, odor like carbon tetrachloride at high concentrations.
Vapor pressure:	363.6 mm Hg at 25°C
Solubility:	Practically insoluble in water (0.017 g/100 g water at 25°C). Soluble in alcohol, benzene and ether.
Conversion factor:	7.66 μg/m^3 per ppb at 25°C

III. Major Uses and Sources (HSDB, 1995)

The reported total demand for all chlorofluorocarbons (CFCs) in the USA in 1985 was 458,000 tons (WHO, 1990). The three major CFCs in 1985, Freon 11, Freon 12 and Freon 113, accounted for 83% of the total CFCs produced in the USA. The major uses of 1,1,2-trichloro-1,2,2-trifluoroethane (Freon 113) are as a solvent for degreasing and dry cleaning, refrigerant, component in fire extinguishers, chemical intermediate and a foam blowing agent. Freon 113 is not used in aerosol formulations as a propellant component. Its commercial use has become increasingly restricted since 1977 because of the likelihood that CFCs, particularly saturated CFCs such as Freon 113, are depleting the stratospheric ozone layer around the earth. According to the Montreal Protocol, fully halogenated CFC production in industrialized countries should have ended by Jan. 1, 1996. Freon 113 may be released to the environment as emissions from production, storage, and transport; from turbine engine exhaust; by its use as a foaming agent, refrigerant, and solvent; or by its use in the manufacture of fluoropolymers. Human exposure is predominantly by inhalation. The general population is exposed to Freon 113 in ambient air.

IV. Effects of Human Exposures

The nature of the known human health effects primarily involve impairment of neurological and cognitive functions and of the cardiovascular system following acute exposure to levels greatly exceeding the established TLV of 1000 ppm (7700 mg/m^3) (U.S. EPA,1983). Long-term occupational exposure to Freon 113 results in no signs or symptoms of adverse effects. Fifty male workers at the Kennedy Space Center exposed to levels of Freon 113 ranging from 46 to 4700 ppm for an overall average duration of 2.77 years showed no adverse effects (Imbus and Adkins, 1972). In another occupational study, men and women that were exposed to 23 to 62 ppm for periods of 30 min to 5.8 hours per week for an average of 8.7 years (women) or 11 years (men) showed no adverse effects (Triebig and Burkhardt, 1978). However, an occupational study found several workers with signs and symptoms of psychoorganic syndrome after heavy exposure to Freon 113 for 2.5-4.5 years (Rasmussen *et al.*, 1988).

Human exposure to Freon 113 is predominantly by inhalation and most of it is rapidly cleared from the body by exhalation, undergoing little or no metabolism (U.S. EPA, 1983). Essentially no Freon 113 is retained by tissues 48 hours after repetitive exposures (Stopps and McLaughlin, 1967). In another human inhalation study, low blood/breath ratios of Freon 113 were noted which were consistent with the low solubility of the chemical in blood (Woollen *et al.*, 1990). Freon 113 was not detected in the urine. Individuals with less body fat retained less of the compound following exposure. Only 2.6 to 4.3% of the dose was recovered unchanged in breath after the exposure period.

V. Effects of Animal Exposures

Freon 113 exhibits a biphasic absorption pattern (Azar *et al.*, 1973). A rapid initial increase in blood levels is followed by a slower increase to maximum concentrations. Freon 113 partitions into the fat until equilibrium or steady-state conditions are reached. Partitioning into the brain and other well perfused organs occurs to a much lesser extent (Savolainen and Pfaffli, 1980). The approximate half-life of Freon 113 diminution from adipose tissue in rats following exposure is 7 to 9 hours.

A wide variety of acute and subchronic inhalation exposures of rodent species to levels of Freon 113 ranging from 2000 to 400,000 ppm has been reported (U.S. EPA, 1983). However, most of these studies caused no adverse effects, even after 90-day exposure of rats to 20,000 ppm (155 mg/m^3) (Trochimowicz, 1984) and dogs to 5000 ppm (40 mg/m^3) (Leuschner *et al.*, 1983).

In a two-year study on Cr1:CD(SD)BR rats, groups of 100 males and 100 females were exposed to 0, 15.3, 76.6 or 153 g/m^3 (approx. 0, 2000, 10,000, 20,000 ppm) for 6 hr/day, 5 days/week (du Pont, 1985; Trochimowicz *et al.*,1988). The common chronic exposure parameters were measured regularly, including body weight, appearance and behaviour, hematology, urinalysis and clinical chemistry values. Comprehensive histopathological examinations were performed on rats in the control group and those exposed to 153 g/m^3. The only treatment-related effects reported were decreases in mean body weight and in body weight gain among females (5-10%

during second year) in the 76.6 g/m^3 group and in both sexes ($\geq$10% during second year) in the 153 g/m^3 group, and a slight, transient increase in serum glucose levels in males in the 153 g/m^3 group. There was no evidence of hepatotoxicity in any of the exposure groups as measured by histopathologic examination and clinical laboratory evaluations.

In an earlier study by Desoille *et al.* (1968), rabbits and rats were exposed to approximately 93 g/m^3 (12,000 ppm) Freon 113 for 2 hrs/day, 5 days/week for 2 years. Exposed animals showed signs of dizziness. No compound-related effects on morphology or any other chronic toxicity parameters were found. Some rat deaths occurred during the study in both the control and exposure groups, but the deaths in the exposed group were not attributed to Freon 113 exposure.

In a limited one-generation reproduction study, rats were exposed by inhalation to 0, 39 or 97 g/m^3 (0, 5000 or 12,500 ppm) Freon 113 for 6 hr/day, 5 days/week for either 10 weeks (males) or 3 weeks (females) (EHC 113, 1990). Each male rat was then paired with two females for 2 weeks during which time exposure was 6 hr/day, 7 days/week. Pregnant females were exposed 6 hr/day during gestation and offspring were followed for up to 4 weeks. There were no adverse effects on any of the standard reproductive indices.

VI. Derivation of Chronic Reference Exposure Level (REL)

Study	du Pont, 1985; Trochimowicz *et al.*, 1988
Study population	100 Crl:CDBR rats/group/sex, 800 total.
Exposure method	Discontinuous whole body inhalation exposure (0, 2000, 10,000 or 20,000 ppm).
Critical effects	Alimentary system (decreased body weight gain)
LOAEL	10,000 ppm (76,600 mg/m^3)
NOAEL	2,000 ppm (15,300 mg/m^3)
Exposure continuity	6 hr/day, 5 days/week
Exposure duration	2 years
Average experimental exposure	357 ppm (10,000 x 6/24 x 5/7)
Human equivalent concentration	357 ppm (gas with systemic effects, based on RGDR = 1.0 using default assumption that lambda (a) = lambda (h))
LOAEL uncertainty factor	1
Subchronic uncertainty factor	1
Interspecies uncertainty factor	3
Intraspecies factor	10
Cumulative uncertainty factor	30
Inhalation reference exposure level	10 ppm (10,000 ppb, 90 mg/m^3, 90,000 μg/m^3)

The most appropriate study for the determination of the REL is a two year toxicity study conducted in rats (du Pont, 1985; Trochimowicz *et al.*, 1988). In this study, the lowest dose

tested (2,000 ppm or 15,400 mg/m3 of 5 days a week, 6 hours per day) was considered by the researchers as a NOAEL, though this dose was associated with a slight but statistically significant increase in liver weights among male rats.The significance of this latter finding is clouded by the development of *Corynebacterium kutscheri* infections during the second year of the study, especially since the infection was considered to be more severe in males.

At the 10,000 and 20,000 ppm level, the main effects noted were slight decreases in body weight gain. The magnitude of this effect was less than 10% in the 10,000 ppm group, but greater than 10% in the 20,000 ppm. The biological significance of deficits in body weight gain of less than 10% has been questioned.

Given the complicating infection in male rats, the apparent shallow dose response relationship and mild effects over the range of 2,000 to 20,000 ppm, 2,000 ppm is considered as a NOAEL in this report.

The human and animal studies indicate that Freon 113 is not metabolized once absorbed into the body and is rapidly excreted via the lungs; Freon 113 appears to remain inert within mammalian systems. Occupational studies found no adverse effects with chronic exposure to Freon 113 while human exposure studies found only mild effects.

Strengths of the REL include the availability of long-term inhalation exposure studies in animals. Weaknesses in the database for Freon 113 include the lack of long-term human exposure inhalation studies. A multi-generation study (2 or more generations) would also enhance the database.

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CHRONIC TOXICITY SUMMARY

1,1,2-TRICHLOROETHANE

(*Ethane trichloride; Trichloroethane; 1,2,2-Trichloroethane; β-Trichloroethane; Vinyl trichloride*)

CAS Registry Number: 79-00-5

I. Chronic Toxicity Summary

Inhalation reference exposure level	**400 μg/m^3**
Critical effect(s)	Fatty degeneration and cloudy swelling in livers of rats (inhalation) Decreased liver and kidney weights, decreased liver P450 and aniline hydroxylase activity, decreased prothrombin time and elevated fibrinogen in mice (oral)
Hazard index target(s)	Alimentary system; kidney; nervous system; cardiovascular system

II. Physical and Chemical Properties (Lewis, 1992)

Description	Colorless liquid
Molecular formula	$C_2H_3Cl_3$
Molecular weight	133.4 g/mol
Specific gravity	1.44 @ 20°C
Boiling point	113-114°C
Vapor pressure	23 mm Hg @ 25°C
Solubility	moderately soluble in alcohol and acetone; soluble in chloroform, ethyl ether
Conversion factor	1 ppm = 5.46 mg/m^3 @ 25°C

III. Major Uses and Sources

1,1,2-Trichloroethane is used as an intermediate in the production of vinylidene chloride, as a solvent, and as a component of adhesives (IARC, 1979). It is also used as a solvent in the production of Teflon tubing, in lacquer, and coating formulations (Lewis, 1992), and as a solvent in pharmaceutical manufacture (IARC, 1991).

IV. Effects of Human Exposures

No epidemiological studies were located that specifically examined the effects of 1,1,2-trichloroethane in humans. There have also been no short-term, controlled experimental exposures to this compound in humans.

Twenty-eight percent of 110 sudden deaths in glue-sniffers were associated with trichloroethane (Ellenhorn, 1988). While the exact isomer was not specified, presumably it is the 1,1,1- isomer.

V. Effects of Animal Exposures

The acute toxicity of 1,1,2 -TCA is several times that of the 1,1,1- isomer (Torkelson, 1994). The liver is a primary target organ for chronic 1,1,2-TCA toxicity. The central nervous system appears to be a critical target upon acute exposures.

Female mice treated with doses of 0, 3.9, 44, or 384 mg 1,1,2-TCA/kg in the drinking water for 90 days showed increased serum levels of SGPT, SGOT, and serum alkaline phosphatase (SAP) activity (White *et al.*, 1985). Liver microsomal cytochrome P450 content and aniline hydroxylase activity were also decreased in female mice treated with 44 mg/kg. At the 384 mg/kg dose, there was an elevation in absolute and relative liver and spleen weights and a decrease in brain weight. Several hematological parameters were affected, including decreased prothrombin time and increased plasma fibrinogen. Male mice appeared to respond slightly differently; absolute liver and kidney weights were significantly decreased with exposure to 46 or 305 mg/kg. However, these decreases were not significant when considering proportion of body weight. Water consumption was significantly lower in the high-dose males. No adverse effects were noted at 4.4 mg/kg in the males.

Mice (50 per sex per group) were given daily oral gavage doses of 1,1,2-TCA 5 days per week for 78 weeks (NCI, 1978). Mice were treated with 0, 150, or 300 mg/kg per day for 8 weeks and then 0, 200, and 400 mg/kg/day for 70 weeks. Twelve to 13 weeks were then allowed to elapse without treatment before termination of the experiment. The time weighted average doses were 195 and 390 mg/kg per day (averaged over a 7 day per week period). No significant treatment-related non-neoplastic histological lesions were recorded in surviving animals.

Similar negative results were observed in a companion study by NCI (1978). In this study rats were treated with 0, 46, and 92 mg/kg/day (time weighted average, calculated in an analogous fashion to the mouse study) for 78 weeks.

The acute oral LD_{50}s for these mice were 378 and 491 mg/kg for the male and female mice, respectively (White *et al.*, 1985). Most of the deaths occurred within 24 hours of exposure, with animals showing signs of severe CNS toxicity in form of sedation and loss of righting reflex. Necropsies showed gastric irritation, pale livers, and a number of reddened or hemorrhagic areas in the lungs.

Torkelson (1994) describes unpublished data by Dow Chemical Company in which daily 7-hour exposures to 15 ppm, 5 days/week for 6 months were without adverse effects in rats (Dow Chemical Company, 1963, cited in Torkelson, 1994). The specific endpoints examined in this study were not completely reported. Fatty degeneration and cloudy swelling was seen in livers from rats after 16 seven-hour exposures to 30 ppm. However, in this study, the single 7-hour LC_{50} was reportedly 500 ppm. The actual data from Dow Chemical Company were not available for review.

VI. Derivation of Chronic Reference Exposure Level

Derivation of Chronic Inhalation Reference Exposure Level

Study	Torkelson (1994)
Study population	Rats
Exposure method	Discontinuous whole-body inhalation
Critical effects	Fatty degeneration and cloudy swelling in livers
LOAEL	30 ppm
NOAEL	15 ppm
Exposure continuity	7 hours per day
Exposure duration	16 days (LOAEL group) 6 months (NOAEL group)
Average experimental exposure	15 ppm for NOAEL group
Human equivalent concentration	24 ppm (gas with systemic effects, based on RGDR = 1.6 for lambda (a) : lambda (h) (Gargas et al., 1989))
LOAEL uncertainty factor	1
Subchronic uncertainty factor	1
Interspecies uncertainty factor	3
Intraspecies uncertainty factor	10
Database uncertainty factor	10 (Incomplete documentation of all chronic inhalation studies)
Cumulative uncertainty factor	300
Inhalation reference exposure level	0.08 ppm (80 ppb; 0.4 mg/m^3; 400 $\mu g/m^3$)

Few toxicological investigations in humans or laboratory animals have been conducted for 1,1,2-TCA. There are no published inhalation studies, and 2 subchronic oral exposure studies. The only subchronic inhalation study for 1,1,2-TCA (Dow, unpublished data cited in Torkelson, 1994) was not available for review upon request. Therefore additional uncertainty arises from an inability to evaluate the original study data.

The strengths of the inhalation REL include the availability of subchronic to chronic inhalation exposure data and the observation of a NOAEL. Major areas of uncertainty are the lack of adequate human exposure data and the lack of reproductive and developmental toxicity studies.

NCI (1978) examined the effects of daily gavage of high concentrations of 1,1,2-TCA on tumor formation in mice and rats. Although mortality rates were high over the course of the study in all groups, no specific treatment-related non-neoplastic effects were observed. The focus of the NCI study was on carcinogenic effects, the doses used were relatively high, and the route of exposure (oral gavage) and dosing regimen may have resulted in undue complicating stress on the animals over a subchronic exposure. In contrast, the study by White *et al.* (1985) used a wide range of doses, given in drinking water for a more continuous exposure, and a battery of non-carcinogenic effects were measured. Although statistically significant increases in SGOT and SAP were observed in the females at all doses, the increase was slight, and without a dose-response. Therefore, the 3.9 mg/kg dose was considered a NOAEL.

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CHRONIC TOXICITY SUMMARY

TRICHLOROETHYLENE

(trichloroethylene; 1,1-2-trichloroethylene, 1,1-dichloro-2-chloroethylene, acetylene trichloride, and ethylene trichloride (Mycroft and Fan, 1985))

CAS Registry Number: 79-01-6

I. Chronic Toxicity Summary

Inhalation reference exposure level	**600 µg/m^3**
Critical effect(s)	Neurotoxicological effects (drowsiness, fatigue, headache) and eye irritation in workers.
Hazard index target(s)	Nervous system; eyes

II. Physical and Chemical Properties (Fan, 1988)

Molecular formula	C_2HCl_3
Molecular weight	131.4
Description	Colorless vapor; sweetish, chloroform-like odor
Specific gravity	1.47 (water = 1)
Boiling point	87.7°C
Vapor pressure	77 mm Hg @ 25°C
Vapor density	4.5 (air = 1)
Solubility	Soluble in alcohol, ethers, petroleum distillates and other halogenated solvents
Conversion factor	1 ppm = 5.37 mg/m^3 @25° C

III. Major Uses or Sources

Trichloroethylene was once used as an extractant in food processing and has been used as an anesthetic and analgesic for medical purposes (Waters *et al.* 1977). Currently, it is widely used as a solvent in the industrial degreasing of metals, with secondary solvent uses in adhesive paint and polyvinyl chloride production (U.S. EPA, 1985). Trichloroethylene is used as a solvent in the textile industry, as a solvent for adhesives and lubricants and as a low-temperature heat transfer fluid (IARC, 1979). Trichloroethylene is also implemented in the manufacturing of pesticides and other chemicals (Feldman, 1979).

IV. Effects of Human Exposure

An occupational study of trichloroethylene (TCE) vapor emissions in a pump room was conducted by Vandervort et al. (1973). Workers were an average age of 40 and had been employed for an average of 8 years. For 11 day shift workers, individual 8 hour time weighted averages (TWAs) of TCE exposure concentrations were extrapolated from two area samples, and these averages ranged from 170-420 mg/m^3 (32-78 ppm). Nineteen workers (including the 11 workers whose work areas were sampled) completed a questionnaire and reported the following symptoms: 73% eye irritation, 70% drowsiness, 58% heart palpitations, 58% cough, 53% weakness and 52% dizziness. Fifty percent of the 19 exposed workers reported that consumption of small amounts of alcohol outside of work resulted in changes of skin color and severe intoxication. One worker of the 19 reported no adverse effects from the occupational exposure. Nine control workers experienced none of the above symptoms. Urine samples from the 19 exposed and 9 unexposed workers were collected before and after the work shift and examined for the TCE metabolites trichloroacetic acid (TCA) and trichloroethanol (TRI). TRI levels ranged from 4-260 mg/l and TCA levels ranged from 4-197 mg/l. Results of the urine assays showed a range of TCE metabolite concentrations and therefore, confirmed that the workers were exposed to a variety of concentrations in their environments.

Nomiyama *et al.* (1977) examined 36 trichloroethylene workers, of which 9 males and 12 females were occupationally exposed to a constant concentration of trichloroethylene (TCE) and 18 males were exposed to variable concentrations (duration of exposure unspecified). The control group consisted of 6 males and 10 females who were of similar educational, sociologic and economic status to the trichloroethylene workers. Researchers used urinary excretion of TCE metabolites as an indicator of the level of TCE exposure in the working environment; total excreted trichloro-compounds of 100 mg in 4 hours corresponding to 100 ppm TCE present in the working environment (Bardodej, 1958; Medek, 1958). Of the 36 exposed workers, 5 were exposed to 0-25 ppm, 14 were exposed to 25-50 ppm, 6 were exposed to 50-100 ppm, 8 were exposed to 100-150 ppm and 3 were exposed to 150-200 ppm TCE. In the low exposure group, workers experienced mucous membrane irritation in the eyes, nose and throat, in addition to drowsiness, fatigue and headache. These symptoms were persistant through the higher concentration exposures with an increase in eye irritation, headache, fatigue, and nasal obstruction above 100 ppm TCE. Increases in Rhinorrhea and drowsiness were seen above 50 ppm TCE exposure.

Kimmerle *et al.* (1973) exposed 4 human subjects (3 males and 1 female) to a subacute regimen of 48 $\pm$ 3 ppm trichloroethylene (TCE) for 4 hours a day over a period of 5 days. Levels of TCE and the metabolites trichlororethanol (TRI) and trichloroacetic acid (TCA) were determined. Trichloroethanol-blood levels were elevated immediately after exposure, and detection of trichloroethanol occurred up to 7 days after the last exposure. TCE-blood concentration increased slightly over the 5 days. Levels of urinary excreted trichloroethanol, as well as the TCA-concentration, increased throughout the study, with the female showing a significantly higher TCA-excretion. Levels of TCA were found up to 12 days after the final exposure.

Okawa *et al.* (1973) studied the occupational exposure of 24 electrical plant workers to trichloroethylene (TCE). The plant worker group consisted of 22 males and 2 females ranging in age from 21-52 years old. Environmental samples of TCE were collected over three days and yielded varying concentrations of TCE related to the task performed in certain areas (duration of exposure unspecified). Spray booth operators were exposed to an average of 25.3 ppm TCE (13-40 ppm range) in addition to averages of 15.2 ppm n-propyl acetate (NPA) and 6 ppm toluene (TOL). Workers involved in washing board units were exposed to an average value of 39 ppm (6-82 ppm range) TCE. Though the workers wore respiratory protection during the washing proceedure, the overall average of airborne TCE in this area was 48.3 ppm. In the testing area of the plant, researchers report that the amounts of TOL and NPA were insignificant. Here, TCE levels were an average of 24.4 ppm (range = 8-44 ppm). The solder machine operators were exposed to an average of 44.0 ppm TCE (range = 23-87 ppm) with no NPA or TOL present. During the cleaning of the soldering machines, TCE levels rose to an average of 70.5 ppm (range = 30-106 ppm). Concentrations were only at these elevated levels for 20-30 minutes a day. Researchers note that although other agents were used in the work area, TCE was the only chemical found in significant amounts throughout the work area and that the levels of NPA and TOL were insignificant. An anaylsis of urinary TCE metabolites indicating that the workers were exposed to a time weighted average concentration of <50 ppm TCE. Three of the 24 workers reported that they were unaffected by their working conditions, but the most prominent complaints consisted of 70.8% workers experiencing nausea, 54.2% headache, 33.3% dizziness, 25.0% fatigue, 25% nose and throat irritation and 20.8% eye irritation. Workers reported that these symptoms were alleviated hours after leaving the work environment. Researchers collected 8 hour urine samples from 20 of the workers and from 9 controls and analyzed them for TCE metabolites. Results of urinary analysis showed that the controls had exposure to an unspecified amount of TCE. TCA levels in exposed workers were elevated from that of the controls and correlated to the different exposures in specific work areas.

Phoon *et al.* (1984) reported on 5 cases of Stevens-Johnson syndrome (erythema multiforme major) with liver involvement which followed exposure to TCE. In two cases, reactions to the exposure began with a fever followed by an itchy rash on the face spreading over the body. Lesions were observed on the face, arms and in the mouth. Liver function tests were abnormal. One of the two developed jaundice with hepatomegaly. Case #3 developed a similar reaction after 5 weeks of exposure to 216-912 mg/m^3 TCE (40-170 ppm) as did case #5 after two weeks of exposure to 370 mg/m^3 TCE (69 ppm). Case #4 involved a 39 year old man exposed to <50†mg/m^3 TCE (< 9.3 ppm) for three weeks who developed the characteristic rash, lesions and jaundice with slight hepatomegaly. Upon returning to work over the next three weeks, he developed generalized erythrodermia and facial oedema, hepatosplenomegaly and liver failure with septicemia from which he died 14 days later.

Stewart *et al.* (1974) studied the effects of subacute trichloroethylene (TCE) exposure in combination with alcohol consumption. Seven men exposed to 200ppm TCE ingested 1 qt of beer or 90 ml of 100-proof vodka and developed red blotches on their faces 30-40 minutes later. These lesions enlarged with time until they reached a peak intensity, whereupon they faded. One subject experienced facial flush with the consumption of alcohol for three weeks after the last TCE exposure, while another showed flushing six weeks after the last exposure.

V. Effects of Animal Exposure

Kjellstrand *et al.* (1983) studied the effects of both intermittent and continuous exposures of various concentrations of trichloroethylene on male and female mice over a period of 30 days. The concentrations used range from 37-3600 ppm, and 7 of the 14 groups were continuously or intermittently exposed to lower concentrations of 37, 75, 150, 225 and 300 ppm TCE. Continuous exposure studies were conducted over a period of 30 days for exposure groups of 37, 75, 150 and 300 ppm TCE. All groups consisted of 10 males and 10 females (except the 37 ppm group, consisting of 20 males and 20 females) and were compared to identical groups of air-exposed controls. Liver weights increased in a non-linear fashion as the concentration level of TCE increased. All groups exhibited statistically significant increases in liver weights as compared to the controls. In both the 37 and the 75 ppm groups, the increase in females was less than in males. No increase in spleen weight was detected at either the 37 or 75 ppm exposure level. At the 37 ppm level, a slight increase in plasma butyrylcholinesterase (BuChE) activity (not statistically significant) was also detected. A significant increase in kidney weight was seen in the male 75 ppm group and was more pronounced with increasing concentration. Male mice in the 75 ppm group also showed statistically significant increases in BuChE activity. In the 150 ppm group, male and female liver weight increases were statistically significant and of equal magnitude. A statistically significant increase was seen in the BuChE activity of the 150 ppm male mice. It was not until female mice were exposed to 300 ppm, that they showed slight increase in BuChE activity, while the males increased 3.5 times the controls. Liver weight increases for the 300 ppm group were close to the maximum with females showing greater increase than the males. Ten male and 10 female mice were continuously exposed to 150 ppm TCE for 30 days, but then allowed a 120 day rehabilitation period. Following rehabilitation, liver weights returned to levels comparable to the controls. The elevated BuChE activity returned to a normal level. No significant effects were seen after the period of rehabilitation. A continuous study was performed on 10 male and 10 female mice for 120 days at an exposure level of 150†ppm TCE. No further increase in liver weight occurred beyond the level reached in the 30 day study. Body weight gain was slightly decreased, and the same level of BuChE activity was seen as in the 30 day exposure. The intermittent study consisted of 30 days exposure to 225 ppm TCE for 16 hours a day, 7 days a week. A significant increase was seen in the BuChE activity of male mice, while females did not exhibit an increase in BuChE activity. Both males and females showed statistically significant increases in liver weight. Kidney weight increased in the same manner as in the continuous exposures. The authors noted that “extrapolation of the concentration-effect curve suggests that both liver weight and BuChE activities are influenced at still lower concentration.

Briving *et al.* (1986) examined neurotoxicity as a result of chronic trichloroethylene (TCE) inhalation exposure. Two groups of gerbils (6 in each group) were exposed to 50 or 150 ppm TCE for a period of 12 months. Two equivalent groups were used as controls. Two areas of the brain were specifically observed, the hippocampus and the posterior part of the cerebellar vermis. These discrete brain areas were previously shown to be sensitive towards chlorinated aliphatic solvents (Haglid *et al.*, 1981). Following exposure, gerbils were decapitated and measurements were made of total free tissue amino acids as well as high-affinity uptake and release of ^{3}H-

aminobutyric acid (GABA) and ^{14}C-glutamate. A significant increase in glutathione was seen in the hippocampus of the 150 ppm gerbils, but amino acid levels were not significantly affected. In the posterior part of the cerebellar vermis, glutamate and GABA accumulation levels increased in a dose-dependent manner, with significant increase seen at both 50 and 150 ppm TCE. Evaluation of the hippocampus revealed no significant changes. Authors suggest that the stimulation of transport functions for GABA and glutamate may be triggered by the presence of the TCE metabolite, trichloroethanol. Therefore, the levels of GABA and glutamate are indicative of the amount of trichloroethanol from TCE in the brain.

Kligerman *et al.* (1994) exposed 20 male CD rats to 0, 5, 50, or 500 ppm trichloroethylene (TCE) for 6 hours a day, over a period of 4 days. Groups at each concentration consisted of 5 rats. One of the cytogenoic effects measured was abnormal peripheral blood lymphocytes (PBLs), with regard to sister chromatid changes. Also analyzed, were the cell cycle, bone marrow micronuclei in polychromatic erythrocytes (MN-PCEs/1000) and micronuclei in cytochalasin B-blocked binucleated cells (MN-BN/1000). The 5 ppm and 500 ppm exposure groups showed a decrease (not statistically significant) in cell cycle. In addition, the 50 ppm group exhibited a statistically significant decrease in cell cycle. For all concentrations, there was an overall increase in the PCE percentage. The number of micronuclei PCEs also rose with the increasing concentrations of 50†ppm and 500 ppm TCE (not statistically significant due to high control values). Researchers conclude that the resulting increase of MN in exposed rats is indicative of aneuploidy induction as opposed to chromosomal breakage, and that the lack of chromosome aberrations corresponds to spindle effects such as aneuploid induction. Concurrent results of increased levels of leukocyte aneuploidy were also found by Konietzko *et al.* (1978) in degreaser workers occupationally exposed to TCE.

Haglid *et al.* (1981) continuously exposed gerbils to 60 ppm or 320 ppm trichloroethylene (TCE) for 3 months. Following the exposure period, gerbils were maintained for 4 months in TCE-free conditions in order to observe any restoration of neuronal function. Both of the exposed groups as well as the control group consisted of six pairs of males and females. Brain samples were collected from the gerbils after the 4 month non-exposure period and used for determination of DNA and proteins. In order to determine areas of the brain that were sensitive to TCE, researchers examined biochemical and morphological changes in the hippocampus, the posterior part of the cerebellar vermis and the brain stem. In addition to the biochemical tests, the cerebellum, brain stem and cerebrum of two gerbils from each group, including the control, were used for neuropathological examination. Brain tissue from 2 gerbils in the control group and the 320 ppm group were examined under the electron microscope. No difference was seen in the body and brain weights of the exposed gerbils compared with controls. A slight but significant increase in soluble proteins was detected in the frontal cerebral cortex of the 60 ppm group, and a more significant elevation was seen in the visual cerebral cortex of both the 60 ppm and 320 ppm groups. In the 60 ppm group, a slight but significant decrease was seen in the soluble proteins of the sensory-motor cortex. Both groups exhibited significant decreases in levels of soluble proteins in the hippocampus, the brain stem and in the posterior part of the cerebellar vermis. Soluble protein levels in the cerebellar hemisphere and anterior part of the vermis of gerbils in both exposed groups did not differ from the controls. The 320 ppm group showed significantly increased DNA levels in the posterior part of the sensory motor cortex and cerebellar vermis.

The glial cytoplasmic protein (S 100 fraction) level of the 60 ppm group was decreased in the frontal and visual cerebral cortex, but increased in the posterior part of the cerebellar hemisphere and the sensory-motor cortex. However, only a slight decrease of S 100 protein was observed in the visual cerebral cortex of 320 ppm exposed gerbils. Most notable S 100 increase occurred in the hippocampus, brain stem and the posterior part of the cerebellar vermis, indicating that either the glial cells were directly effected or that damage to surrounding neuronal cells caused an indirect response. There was an increase in DNA in the posterior part of the cerebellar vermis in the exposed gerbils, suggesting TCE induced astroglial cell mitosis. Light microscopy revealed shrinkage of cell bodies and axon swelling occurring in various parts of the brain. The electron microscopy performed on control and 320 ppm brain tissues revealed increased levels of filament bundles in the cytoplasm of some Purkinje and Golgi cell perikarya, lysosomes, myelin bodies and lipid containing lysosomal structures in the exposed gerbils. Unique arrangements of filament bundles were seen in Purkinje and Golgi cell dendrites of the exposed group. A significant decrease in the number of microtubules was observed as well as a decrease in the number of synaptic vessicles in the granular layer. Also, the granular layer had decreased maxim al nerve cell surface area. Nerve cells were effected by the exposure as several types were reduced in size with fewer organelles and more lysosomes and myelin bodies. Many axons and dendrites had reduced numbers of microtubles, and there were filament bundles observed that were not present in the controls. Lysosmal structures were increased in the synaptic terminals.

Kimmerle *et al.* (1973) performed a subchronic study on 20 male rats for a period of 14 weeks. Rats were exposed to a mean concentration of 55.0 $\pm$4 ppm trichloroethylene (TCE) for 8 hours a day, 5 days a week. The control group consisted of 20 rats who were housed in similar inhalation chambers under similar conditions to that of the exposed rats. Ten exposed rats were analyzed for TCE metabolite excretion on a daily basis. Blood levels of trichloroacetic acid (TCA), trichloroethanol (TRI) and chloral hydrate (CH) were measured during the 2nd, 3rd, 4th, 6th, 9th and 14th weeks. Weekly measurements of body weights were recorded. Macroscopic examinations were performed on the thyroid gland, heart, lungs, liver. kidneys, testes and adrenal glands. Hematological evaluations, liver function tests and renal function tests were also conducted following exposure. Urinary levels of TRI varied individually among the rats, but a continuous increase in TRI was observed through the 10th week. TCA levels remained fairly constant throughout the duration of the experiment. TCE was not detectable in the blood or the tissues of exposed rats. Although liver and renal function tests did not reveal abnormalities, there was an increase in the liver weights of the exposed rats. The weights of the other organs examined were similar to the controls.

Norpoth *et al.* (1974) observed an increase in liver cytochrome P_{450} activity in 9 rats exposed to 50 ppm trichloroethylene for 28 days, compared with 9 control rats.

VI. Derivation of Chronic Reference Exposure Level

Study	Vandervort *et al.* (1973)
Study population	28 workers
Exposure method	Discontinuous occupational inhalation exposure
Critical effects	Drowsiness, fatigue, headache, and eye irritation
LOAEL	32 ppm (170 mg/m^3)
NOAEL	Not observed
Exposure continuity	8 hours a day (10 m^3/day occupational inhalaton rate), 5 days a week
Exposure duration	8 years
Average occupational exposure	11.4 ppm for LOAEL group
Human equivalent concentration	11.4 ppm for LOAEL group
LOAEL uncertainty factor	10
Subchronic uncertainty factor	1
Interspecies uncertainty factor	1
Intraspecies uncertainty factor	10
Cumulative uncertainty factor	100
Inhalation reference exposure level	0.1 ppm (100 ppb; 0.6 mg/m^3; 600 $\mu g/m^3$)

The Vandervort *et al.* (1973) study accounted for 8 years of human occupational exposure to TCE vapors. Sensitive, non-specific neurotoxicological endpoints were examined and exhibited in a majority of those workers exposed. Although the time-weighted averages (TWAs) included a wide range of concentrations, the TWA of 32 ppm (170 mg/m^3) was shown to contribute to the high incidence (52 - 73%) of adverse effects experienced by the workers. Many of the symtpoms reported by the workers may have been due to short-term fluctuations in the concentrations in the workplace. The symptoms were not reported separately for the various TWAs, therefore, the lowest TWA (32 ppm) was chosen as a LOAEL. Uncertainty includes the small number of workers studied, the limited extent of the effects mentioned, and the lack of a NOAEL. Strengths include the use of human data, the demonstration of a dose-response relationship, and exposure estimates correlated with urinary excretion measurements.

This study was the best chronic account of the non-carcinogenic effects of TCE on humans, but several other studies show similar results. Nomiyama *et al.* (1977) found similar endpoints of drowsiness, fatigue and eye irritation in 36 workers occupationally exposed to trichloroethylene. Okawa *et al.* (1973) also saw non-specific neurological endpoints in 24 electrical plant workers who were similarly exposed to TCE.

The strengths of the inhalation REL include the use of human exposure data from workers exposed over a period of years. Major areas of uncertainty are the lack of reproductive and developmental toxicity studies and the lack of observation of a NOAEL.

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CHRONIC TOXICITY SUMMARY

TRICHLOROFLUOROMETHANE

(Freon 11; fluorocarbon-11; Fluorochloroform; FC 11; CFC-11;)

CAS Registry Number: 75-69-4

I. Chronic Toxicity Summary

Inhalation reference exposure level	**20,000 μg/m^3**
Critical effect(s)	Histopathological changes in the brain, liver, lungs, and spleen in rats
Hazard index target(s)	Nervous system; alimentary system; respiratory system; blood

II. Chemical Property Summary (HSDB, 1995)

Molecular formula	CCl_3F
Molecular Weight	137.38 g/mol
Description	Colorless liquid below 23.7°C; nearly odorless, odor mild and somewhat ethereal at high concentrations.
Vapor Pressure	Approximately 796 mm Hg at 25°C.
Solubility	Very low solubility in water (1.0 g/l water @ 25°C). Soluble in alcohol, ether and other organic solvents.
Conversion factor	5.62 μg/m^3 per ppb at 25°C

III. Major Uses and Sources

The reported total demand for all chlorofluorocarbons (CFCs) in the USA in 1985 was 458,000 tons (EHC 113, 1990). The three major CFCs, trichlorofluoromethane (Freon 11), dichlorodifluoromethane (Freon 12) and trichlorotrifluoroethane (Freon 113), accounted for 83% of the total CFCs produced in the USA in that year. However, many of Freon 11's uses are becoming increasingly restricted or banned (particularly as an aerosol propellant) due to its action as a stratospheric ozone depleter. According to the Montreal Protocol, fully halogenated CFC production in industialized countries should have ended by Jan. 1, 1996. Major uses of Freon 11 include use as a blowing agent in the production of polyurethane foams, refrigerant, degreasing agent, solvent, fire extinguishing agent, aerosol propellant and chemical intermediate. Freon 11 production dropped 74% in 1994 due mainly to its replacement by hydrochlorofluorocarbons, such as HCFC 141b, which have a much lower ozone-depleting potential (C&EN, 1995). All of

the Freon 11 that is produced will eventually be released to the environment as emissions. General population exposure occurs by inhalation in ambient air. Occupational exposure occurs via inhalation and dermal contact.

IV. Effects of Human Exposures

The kinetics and metabolism of CFCs, including Freon 11, are characterized by rapid pulmonary absorption and distribution. There is no indication of any accumulation. Metabolic transformation of Freon 11 and other similar CFCs is negligible, if it occurs at all. Therefore, toxic effects of metabolites are very unlikely. Regardless of the route of entry, CFCs appear to be eliminated almost entirely through the respiratory tract (EHC 113, 1990; Mergner *et al.*, 1975).

Few studies of the chronic toxicity of Freon 11 in humans have been performed. Human exposure to 1000 ppm of Freon 11, 8 hr/day, 5 days/week for a total of 18 exposures had no untoward subjective effects and caused no changes in electrocardiogram or pulmonary function tests (Stewart, 1978). However, minor decrements in cognitive tests were seen in some of the subjects.

V. Effects of Animal Exposures

The pharmacokinetics of CFCs in laboratory animals, including Freon 11, are similar to that in man (EHC 113, 1990; Blake and Mergner, 1974).

In subchronic inhalation studies, exposure to 143 g/m^3 (25,000 ppm) 3.5 hr/day, 5 days/week for 4 weeks resulted in no adverse effects in rats and guinea pigs (Scholz, 1962). Leuschner *et al.* (1983) exposed groups of dogs to 28.5 g/m^3 (5000 ppm) and rats to 57.1 g/m^3 (10,000 ppm) for 6 hr/day for 90 days and found no adverse effects. Clayton (1966), however, reported pathological changes in the brain, liver, lung and spleen of all rats observed following exposure to 68.5 g/m^3 (12,000 ppm), 4 hr/day for 10 days.

Jenkins et al. (1970) investigated the toxic effects of FC-11 in rats, dogs, monkeys and guinea pigs. Animals were exposed to FC-11 (99.98% purity) via inhalation either continuously at 1,008 ± 44 ppm for 90 days, or repeatedly at 10,250 ±100 ppm for 8 hours/day, 5 days/week, for 6 weeks. Animals used for both exposure regimens were NMR1:0(SD) Sprague-Dawley rats (8 males and 7 females), beagle dogs (2 males), and squirrel monkeys (9 males). NMR1:(ASH) Princeton-derived guinea pigs (8 males and 7 females) were used in the continuous exposure study and Hartley guinea pigs were used in the repeated exposure study. Tissues were examined for gross lesions, and histological examinations were performed on several organ tissues of several organs taken from all of the dogs and monkeys and from half of the rats and guinea pigs. No significant differences between pre-and post-exposure values in hematological and biochemical parameters were noted in any species except the dog. Dogs that received continuous or repeated exposures had respective elevations of 51% and 47% in serum urea nitrogen levels

(significant according to the Student t test, $p<0.05$). The investigators did not associate this change with any specific organ or system injury. No consistent histopathological changes were noted.

In a comprehensive long-term oral toxicity study carried out by NCI (1978) technical grade Freon 11 (97% pure) was administered by gavage in corn oil to 50 Osborne-Mendel rats/group/sex and 50 $B6C3F_1$ mice/group/sex for 78 weeks. The control group for each species consisted of 20 untreated controls/sex and 20 vehicle controls/sex. The time-weighted average high and low doses were, respectively, 977 and 488 mg/kg body wt-day for male rats and 1077 and 538 mg/kg body wt-day for female rats; for mice, 3925 and 1962 mg/kg body wt-day for both sexes. All doses were administered 5 days/week. In both male and female rats, a significant ($P < 0.001$) dose-related acceleration of mortality was noted compared with the vehicle control using the Tarone test. Low incidences (2-6%) of pleuritis and pericarditis were seen in treated rats of both sexes at both dose levels. In mice, only female mice had a significant ($p = 0.009$) dose-related increase in mortality compared with controls using the Tarone test. No other treatment-related effects on weight gain, clinical signs or non-tumor pathology were seen in either rats or mice.

VI. Derivation of Chronic Reference Exposure Level (REL)

Study	Jenkins *et al.*, 1970
Study population	Rats
Exposure method	Continuous whole-body inhalation exposures
Critical effects	Histopathological changes in the brain, liver, lungs, and spleen
LOAEL	1,008 ppm (Jenkins *et al.*, 1970)
NOAEL	Not observed
Exposure continuity	Continuous
Exposure duration	90 days
Average experimental exposure	1,008 ppm
Human equivalent concentration	1,008 ppm (gas with systemic effects, based on RGDR = 1.0 using default assumption that lambda (a) = lambda (h))
LOAEL uncertainty factor	3
Subchronic uncertainty factor	3
Interspecies uncertainty factor	3
Intraspecies factor	10
Cumulative uncertainty factor	300
Inhalation reference exposure level	3 ppm (3,000 ppb; 20 mg/m^3; 20,000 $\mu g/m^3$)

The subchronic studies on Freon 11 recorded little or no adverse effects at g/m^3 levels indicating that Freon 11 has relatively low toxicity, similar to other CFCs such as Freon 12 and Freon 113. Human and experimental animal pharmacokinetic studies with Freon 11 indicate that absorption and metabolism of the CFC among mammals are quite similar.

The major strength of the REL is the observation of a mild LOAEL in a subchronic study with histopathological analysis. The primary weaknesses of the database for Freon 11 are the paucity of human health effects data, the lack of a NOAEL, and the absence of chronic animal studies on reproductive and developmental toxicity.

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CHRONIC TOXICITY SUMMARY

TRIETHYLAMINE

(Diethylaminoethane; Ethanamine; N,N-Diethylethanamine)

CAS Registry Number: 121-44-8

I. Chronic Toxicity Summary

Inhalation reference exposure level	**7 µg/m^3 (** (U.S. EPA RfC) This document summarizes the evaluation of non-cancer health effects by U.S. EPA for the RfC
Critical effect(s)	Squamous metaplasia in lung tissue; necrotic inflammation in nasal tissue in rats.
Hazard index target(s)	Respiratory system; immune system; eyes

II. Physical and Chemical Properties (Nelson and Bull, 1990)

Molecular formula	$C_6H_{15}N$
Molecular weight	101.9 g/mol
Description	Colorless liquid and gas
Specific gravity	0.726 @ 25°C
Boiling point	89.3° C
Vapor pressure	400 mm Hg @ 31.5°C
Solubility	soluble in acetone, benzene and chloroform
Conversion factor	1 ppm = 4.14 mg/m^3 25°C.

III. Major Uses or Sources

Triethylamine (TEA) is primarily used as a cross-linking catalyst in the production of polyurethane foam used in the manufacture of cores for metal castings (Albrecht and Stephenson, 1988). Triethylamine is also used as a catalyst for epoxy resins, and as a corrosion inhibitor for polymers (Nelson and Bull, 1990).

IV. Effects of Human Exposures

Acute, high level triethylamine exposures (20 mg/m^3 (4.8 ppm) for 8 hours) resulted in reversible ocular effects that included corneal swelling and halo vision in 4 out of 5 volunteer subjects (Akesson *et al.*, 1988). A medical examination of workers exposed to a time-weighted average concentration of 13 mg/m^3 (3.1 ppm) showed reversible corneal edema (Akesson *et al.*, 1986).

However, small quantities of dimethylethanolamine, toluene diisocyanate and methylene diphenyl isocyanate were also present in the workplace atmosphere.

V. Effects of Animal exposures

Lynch *et al.* (1990) exposed male and female rats to triethylamine at concentrations of 0, 25, or 247 ppm (0, 103.4, or 1022.2 mg/m^3) for 6 hours/day, 5 days/week for 28 weeks. Endpoints examined included gross and histopathological examination of all major organs, including the lungs, nasal passages, and eyes. Clinical enzyme levels (BUN, ALT, AST, CPK, and creatinine, and hematological values (hemoglobin, RBC count) were also measured. No gross or histological effects in any organ were observed in any group. Clinical and hematological parameters were unchanged with exposure.

In a short-term study by Virginia Chemicals (1987), necrotizing inflammation of the nasal cavity, metaplasia of the trachea and thymic atrophy were observed after 6 hours per day, 10 days exposure to 1000 ppm (4140 mg/m^3). Two of five males and one of five females died from pulmonary edema after the seventh day. Thymic atrophy was noted in 7 out of 10 animals, and all animals exhibited necrotizing inflammation in the nasal epithelium.

Rabbits (12 per group) exposed to 48 or 100 ppm (199 or 414 mg/m^3) triethylamine for 7 hours/day, 5 days/week, for 6 weeks showed concentration-dependent pathology in the eyes, lungs, liver, kidney, and heart (Breiger and Hodes, 1951). The lesions in the 48 ppm group were less severe than those seen in the 100 ppm group. No control animals were included in this study, nor were the specific incidences of histologic effects reported.

A chronic 3-generation reproductive study in rats (10/sex/group) was inconclusive due to excessive mortality in controls (Davison *et al.*, 1965). In this study, rats were exposed to 0, 2, or 200 ppm triethylamine. The third generation of the 200 ppm group was changed to 500 ppm since no effects were noted in the 200 ppm group. Exposure of this group to 500 ppm resulted in decreased body weight, and decreased water consumption.

VI. Derivation of U.S. EPA RfC

Study	Lynch *et al.*, 1990; Virginia Chemicals, 1987; U.S. EPA, 1995
Study population	Rats (10/sex/group)
Exposure method	Discontinuous whole-body inhalation
Critical effects	Nasal paasage inflammation
LOAEL	1,000 ppm (Virginia Chemicals, 1987)
NOAEL	247 ppm (Lynch *et al.*, 1990)
Exposure continuity	6 hours/day, 5 days/week
Exposure duration	10 days (Virginia Chemicals, 1987); 28 weeks (Lynch *et al.*, 1990)
Average experimental exposure	44.2 ppm for NOAEL group
Human equivalent concentration	4.7 ppm for NOAEL group (based on a gas with respiratory effects, RGDR= 0.107)
LOAEL uncertainty factor	1
Subchronic uncertainty factor	10
Interspecies uncertainty factor	3
Intraspecies uncertainty factor	10
Modifying factor	10 (database deficiencies)
Cumulative uncertainty factor	3,000
Reference exposure level	0.002 ppm (2 ppb; 0.007 mg/m^3; 7 $\mu g/m^3$)

The major strength of the triethylamine RfC is the observation of a NOAEL in a controlled exposure experiment. The major weaknesses are the lack of adequate human health effects information, the lack of dose-response data or a LOAEL and NOAEL in a single experiment, and the lack of long-term exposure data.

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CHRONIC TOXICITY SUMMARY

VINYL ACETATE

(1-Acetoxyethylene; acetic acid, vinyl ester; acetic acid, ethenyl ester; VAC; vinyl A monomer; ethenyl ethanoate)

CAS Registry Number: 108-05-4

I. Chronic Toxicity Summary

Inhalation reference exposure level	**200 µg/m^3** (U.S. EPA RfC) This document summarizes the evaluation of non-cancer health effects by U.S. EPA for the RfC
Critical effect(s)	Nasal epithelial lesions in rats and mice
Hazard index target(s)	Respiratory system

II. Physical and Chemical Properties (HSDB, 1994)

Molecular formula	$C_4H_6O_2$
Molecular weight	86.09 g/mol
Description	Colorless liquid
Specific gravity	0.932 @ 20° C
Boiling point	72.7° C
Melting point	-93.2° C
Vapor pressure	115 mm Hg @ 25° C
Solubility	Slightly soluble in water, soluble in ethane, acetone, chloroform; >10% soluble in ethanol and benzene
Conversion factor	1 ppm = 3.52 mg/m^3 @ 25°C

III. Major Uses and Sources

The major use of vinyl acetate monomer is in the manufacture of polyvinyl and vinyl acetate copolymers, which are used in water-based paints, adhesives, paper coatings, and applications not requiring service at extreme temperatures (HSDB, 1994). It is also used in safety glass interlayers and in hair sprays (HSDB, 1994).

IV. Effects of Human Exposures

Deese and Joyner (1969) conducted an occupational study of 21 chemical workers with a mean length of employment of 15.2 years and exposed to a time-weighted average of 8.6 ppm (30.3 mg/m^3) VA. No adverse effects were noted following chest x-ray, electrocardiogram, blood chemistry, and urinalysis. The control group (sample size unspecified) consisted of workers in units not exposed to VA. An additional study was performed by Deese and Joyner (1969) that showed intolerable eye irritation in 3 out of 3 subjects exposed for an unspecified extended period of time to 21.6 ppm (76 mg/m^3) VA. Upper respiratory irritation was also experienced by a majority of 5 subjects. Odor was detected at 0.4 ppm (1.4 mg/m^3) in 3 out of 3 subjects.

V. Effects of Animal Exposures

A 104-week inhalation study in rats and mice (90/sex/group) was conducted using concentrations of 0, 50, 200, or 600 ppm (0, 176, 704, or 2113 mg/m^3) vinyl acetate (VA) (Owen, 1988). The Owen (1988) study was later published by Bogdanffy *et al.* (1994).
Exposures were for 6 hours/day, 5 days/week. Histology was performed on all principal major organs. There was no mortality resulting from these exposures. A close examination of the effects of VA on the lung and nasal passages showed significant lesions in the nasal cavity, bronchi, and lungs of rats exposed to 600 ppm VA. Lesions included olfactory epithelial metaplasia/atrophy and nest-like epithelial folds in the nasal cavity, exfoliation of bronchial epithelium, fibrous intraluminal projections in the bronchi, and pigmented histiocyte accumulation in the lungs. Body weight gain of rats was significantly decreased in the 600 ppm VA group. Rats treated with 200 ppm VA showed some evidence of epithelial atrophy and metaplasia in the nasal cavity. No effects were observed in the rats from exposure to 50 ppm VA.

Mice also exhibited significant histological lesions in the respiratory tract following exposure to 200 ppm VA or greater. The lesions included atrophy of the olfactory epithelium and submucosal gland. At the 600 ppm concentration, hyperplasia of the trachei was observed, in addition to exfoliation/flattening of the bronchial epithelium and decreased body weight gain. Relative brain and kidney weights were increased in the 600 ppm group at the end of the study, and absolute liver, heart and kidney weights were also significantly elevated. No adverse effects were observed in the 50 ppm group.

A 13-week study on the effects of VA in mice was conducted by Owen (1980a). In this study, mice (10/sex/concentration) were exposed to 0, 50, 200, or 1000 ppm (0, 176, 704, or 3520 mg/m^3) VA for 6 hours/day, 5 days/week for 13 weeks. A concentration-dependent increase in the incidence of diffuse rhinitis, beginning a the 200 ppm concentration, was detected using histopathological examination. Focal pneumonitis was observed in the 1000 ppm treatment group. No adverse effects were seen in the 50 ppm treatment group. An identical study in rats was also conducted by Owen (1980b). In this study, body weight gain was significantly reduced in male and female rats exposed to 1000 ppm VA. An increase in the incidence of mild histiocytic alveolitis was observed in the 1000 ppm group.

A study on the developmental toxicity of VA in rats was conducted by Irvine (1980). In this study, groups of 24 pregnant female rats were exposed to 0, 52, 198, or 1004 ppm (0, 182, 696, or 3533 mg/m^3) VA for 6 hours/day on days 6-15 of gestation. Significant maternal toxicity, as measured by reduced weight gain from day 10 through day 15 was observed in animals exposed to 1004 ppm. Fetotoxicity, as measured by reduced crown-rump length, reduced body weight, and increased incidence of ossification defects in the sternebrae and occipital regions was observed in the 1004 ppm group. No maternal or fetal effects were seen at the lower two VA treatments.

VI. Derivation of U.S. EPA RfC

Study	Owen, 1988; Dreef van der Meulen, 1988; Beems, 1988; U.S. EPA, 1995
Study population	Male and female Sprague-Dawley rats and CD-1 mice (90/sex/group)
Exposure method	Discontinuous inhalation exposures (0, 50, 200, or 600 ppm) over 104 weeks
Critical effects	Histological lesions of the nasal epithelium
LOAEL	200 ppm
NOAEL	50 ppm
Exposure continuity	6 hours/day, 5 days/week
Exposure duration	104 weeks
Average experimental exposure	8.9 ppm for NOAEL group
HRC	1.4 ppm for NOAEL group (RGDR = 0.16 based on a gas with respiratory effects)
LOAEL uncertainty factor	1
Subchronic uncertainty factor	1
Interspecies uncertainty factor	3
Intraspecies uncertainty factor	10
Cumulative uncertainty factor	30
Inhalation reference exposure level	0.05 ppm (50 ppb, 0.2 mg/m^3, 200 μg/m^3)

Acetaldehyde, a hydrolysis product of vinyl acetate, was present in the Owen (1988) study at a concentration of 49 ppm (89 mg/m^3). The duration-adjusted concentration for acetaldehyde was 16 mg/m^3, whereas the NOAEL for histological lesions in rats by Appleman *et al.* (1982) was 48.75 mg/m^3. Therefore, the concentration of acetaldehyde was not considered to account for significant irritation in the Owen (1988) study.

The strengths of the inhalation REL include the availability of controlled exposure lifetime inhalation studies in multiple species at multiple exposure concentrations and with adequate histopathogical analysis, and the observation of a NOAEL. The major area of uncertainty is the lack of adequate human exposure data.

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CHRONIC TOXICITY SUMMARY

VINYL BROMIDE

(Bromoethene; bromoethylene; monobromoethylene)

CAS Registry Number: 593-60-2

I. Chronic Toxicity Summary

Inhalation reference exposure level	**7 μg/m^3** (U.S. EPA RfC) This document summarizes the evaluation of non-cancer health effects by U.S. EPA for the RfC
Critical effect(s)	Liver (hypertrophy and eosinophilic and basophilic hepatocellular foci) in rats.
Hazard index target(s)	Alimentary system

II. Chemical Property Summary (HSDB 1995)

Molecular formula	C_2H_3Br
Molecular weight	106.96
Description	Gas under normal conditions, colorless liquid under pressure. Characteristic pungent odor.
Vapor Pressure	1033 mm Hg at 25°C
Solubility	Insoluble in water at 20°C. Soluble in chloroform
Conversion factor	4.4 μg/m^3 per ppb at 25°C

III. Major Uses and Sources

Vinyl bromide is used as an intermediate in organic synthesis and for the preparation of plastics by polymerization and copolymerization (HSDB, 1995). Other uses include production of flame-retardant synthetic fibers found primarily in children's sleepwear and carpeting. It is not known whether vinyl bromide occurs as a natural product. If released to the atmosphere, vinyl bromide is expected to exist almost entirely in the vapor phase. Exposure, via inhalation, may occur in the vacinity of industries and in industrial settings where this compound is manufactured or used.

IV. Effects of Human Exposure

Currently, there are no adequate chronic exposure data in humans (IARC Monographs, 1986). However, because of its chemical similarity to vinyl chloride, may show similar chronic effects at similar exposure levels.

V. Effects of Animal Exposure

Measurable levels of vinyl bromide in the blood were attained in inhalation studies of the rat indicating that the compound is readily taken up by the lung (Benya *et al.*, 1982; Leong and Torkelson, 1970). *In vitro* studies indicate that the liver microsomal system metabolizes vinyl bromide to 2-bromoethylene oxide, a relatively unstable epoxide. The reactive metabolite may then irreversibly bind to proteins and nucleic acids (Bolt, 1988). Vinyl bromide is also thought to act as a suicide substrate for cytochrome P450 (Bolt *et al.*, 1980).

Only one long-term comprehensive inhalation study has been performed with vinyl bromide (Benya *et al.*, 1982; Busey, 1979). This study was primarily concerned with the carcinogenic effects of the compound. Sprague-Dawley rats, 144 control animals/sex and 120 treated animals/sex/group, were exposed 6 hr/day, 5 days/ to 0, 10, 50, 250 or 1250 ppm vinyl bromide for up to 2 years. The actual average chamber concentrations were 0, 9.7, 52, 247, 1235 ppm, respectively. Non-neoplastic histopathology after 18 months exposure was localized to the liver. The non-neoplastic findings included an increase in eosinophilic and basophilic foci at the two lowest dose levels (9.7 and 52 ppm) over controls. These foci are thought to be an early stage in the progression to cancer, but very few of these foci may actually progress to cancer. When exposure to the toxicant is stopped, these lesions can regress. At the highest two exposure levels (247 and 1235 ppm), these lesions were fewer in number than in the controls. Therefore, no dose-dependent increase in hepatocellular foci was found. The report interpreted the small number of lesions at the higher exposure levels to be due to the high incidence of neoplasms in these groups, and the small number of animals surviving to terminal sacrifice. Other chronic effects seen were microcytic anemia, hematuria, liver and kidney weight elevations, dose-related decrease in body weight and increase in mortality. The clinical effects and kidney weight elevation were seen at the highest dose level while the increase in relative liver weight (20%) occurred in all exposed groups of male rats as early as 6 months.

Other vinyl bromide inhalation studies that exist in the literature are subchronic studies. In a 6 month inhalation toxicity study, rats, rabbits and monkeys were exposed to 1100 or 2200 mg/m^3 (250 or 500 ppm) vinyl bromide for 6 hr/day, 5 days/week (Leong and Torkelson, 1970). No significant change was detected in food consumption, hematology, gross pathology or histopathology.

In a study of vinyl bromide exposure (2000 ppm) by newborn Wistar rats 8 hr/day, 5 days/week for up to 15 weeks, ATPase-deficient liver foci developed (Bolt *et al.*, 1979, 1982). However, the extent of foci development was ten-fold lower than was seen after similar exposure to vinyl chloride.

VI. Derivation of U.S. EPA RfC

Study	Benya *et al.*, 1982; Busey, 1979
Study population	120 rats/exposure group/sex and 144 control rats/sex (1248 total animals)
Exposure method	Discontinuous whole body inhalation exposure (9.7, 52, 247 or 1235 ppm)
Critical effects	Liver hypertrophy and hepatocellular eosinophilic and basophilic foci
LOAEL	9.7 ppm
NOAEL	Not observed
Exposure continuity	6 hours/day, 5 days/week
Exposure duration	2 years
Average experimental exposure	1.7 ppm for the LOAEL group
Human equivalent concentration	1.7 ppm for the LOAEL group (gas with systemic effects, based on RGDR = 1.0 using default assumption that lambda (a) = lambda (h))
LOAEL uncertainty factor	10
Subchronic uncertainty factor	1
Interspecies uncertainty factor	3
Intraspecies factor	10
Modifying factor	10
Cumulative uncertainty factor	3,000
Inhalation reference exposure level	0.002 ppm (2 ppb, 0.007 mg/m^3, 7 $\mu g/m^3$)

The long-term vinyl bromide inhalation rat study, consisting of interim reports and a final report, was clearly the most comprehensive animal study performed with this compound (Benya *et al.*, 1982; Busey, 1978; Busey, 1979). Adverse effects were observed primarily in the liver at all exposure levels. Therefore, a NOAEL was not observed in this study.

Other acute and subacute studies support the liver as the primary target organ following inhalation exposure to vinyl bromide (Bolt *et al.*, 1979; Drew *et al.*, 1976). However, a subchronic study with 60 rats, 6 rabbits and 6 monkeys found no exposure-related effects following inhalation of 250 or 500 ppm vinyl bromide for 6 months (Leong and Torkelson, 1970). In animal studies with the structurally similar compound vinyl chloride, the liver is also a sensitive target for both cancer and noncancer endpoints. While there is a lack of human evidence for vinyl bromide toxicity, the human toxicity (including carcinogenicity) of vinyl chloride is well documented (IARC Monographs, 1979). Angiosarcomas, hepatocellular hypertrophy and altered hepatocellular foci are caused by both vinyl chloride and vinyl bromide in animals following long-term inhalation exposure.

Weaknesses of the database for vinyl bromide include the lack of chronic animal studies. Only one species, the rat, has been used in long-term animal studies. A chronic inhalation study with a second species, preferably non-rodent, would enhance the database considerably. In addition, no developmental or reproductive toxicity data have been generated for vinyl bromide. So it is not

known if the liver is the only sensitive indicator of chronic noncancer effects following vinyl bromide exposure. Finally, human data on chronic exposure is virtually nonexistant.

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CHRONIC TOXICITY SUMMARY

VINYL CHLORIDE

(Chloroethene; chloroethylene; vinyl chloride monomer; VC; VCM)

CAS Registry Number: 75-01-4

I. Chronic Toxicity Summary

Inhalation reference exposure level	**5 μg/m^3**
Critical effect(s)	Liver dysfunction in humans
Hazard index target(s)	Alimentary system; nervous system

II. Physical and Chemical Properties (HSDB, 1995)

Description	Colorless gas
Molecular formula	C_2H_3Cl
Molecular weight	62.5 g/mol
Specific gravity	0.91 @ 20°C
Boiling point	-13 to -37°C
Vapor pressure	2660 mm Hg @ 25°C
Solubility	soluble in alcohol, ethyl ether, carbon tetrachloride, benzene
Conversion factor	1 ppm = 2.56 mg/m^3 @ 25°C

III. Major Uses and Sources

The chief use of vinyl chloride (VC) is in the production of polyvinyl chloride (PVC) resins used for plastic piping and conduit (IARC, 1987). It has also been used in the manufacture of methyl chloroform (IARC, 1979). Vinyl chloride was used as a propellant until 1974 when this use was banned due to its demonstrated carcinogenicity. The main toxicological concern for vinyl chloride is from exposure to the monomer rather than to the polymerized forms (i.e. PVC). Thermal decomposition of VC produces hydrogen chloride, carbon monoxide, and traces of phosgene (ACGIH, 1993).

IV. Effects of Human Exposures

Repeated occupational exposures to vinyl chloride have resulted in a vasospastic syndrome in the hands, similar to Raynaud's syndrome, including acro-osteolysis, scleroderma-like skin changes,

dermatitis, and central nervous system symptoms (together classified as "vinyl chloride disease") (CDHS, 1990).

Twelve of 271 vinyl chloride polymerization workers exposed to vinyl chloride monomer for an average of 5.1 years exhibited abnormal liver function tests as measured by serum biochemistry (Ho *et al.*, 1991). The twelve workers with abnormal liver function tests were considered to have vinyl chloride-induced liver dysfunction; they also have abnormal liver scans and abnormal liver biosies. The geometric mean environmental concentration of VC in the plant was 6 ppm (range = 1 to 10 ppm) in 1976 and 1.5 ppm in 1983 (range = 0.6 - 10 ppm). No control group was studied, but no additional cases of liver dysfunction were observed after VC concentrations were reduced to below 1 ppm.

Similarly, a trend for increasing frequency of abnormal liver function tests with increasing VC exposure was found in workers (Liss *et al.*, 1985). Specifically, impaired indocyanine green clearance and elevated serum bile levels were associated with increasing VC exposure.

A study of 705 rubber workers indicated a significant ($p < 0.05$), dose-dependent relationship between vinyl chloride exposure and symptoms of dizziness or light-headedness, muscle weakness, and local irritation of the eyes, nose and throat (Spirtas *et al.*, 1975). Exposures were broadly categorized and determinations of effects at specific concentrations were not done.

V. Effects of Animal Exposure

Rats (male; 6 - 30 per group) were exposed to 0, 10, 100, or 3000 ppm vinyl chloride for 3, 6, 9, 12 or 18 months (Bi *et al.*, 1985). Exposure to 100 or 3000 ppm vinyl chloride resulted in lower body weights throughout the 18-month period. After 3 months, kidney and spleen weights were greater in the 3000 ppm group, while heart weights were greater in the 100 ppm group. At 6-months, testis weights were decreased in rats exposed to 100 and 3000 ppm and liver weights were increased at all treatment levels compared with controls. Dose-dependent damage to seminiferous tubules was seen at the 100 and 3000 ppm concentrations. Increased liver, spleen, and heart weights were observed in the 10 ppm group at 18 months.

A 10-month exposure of rats (80 per group) to VC concentrations of 0, 50, 500, or 20,000 ppm resulted in increased heart and spleen weights, decreased body weights, and increased serum protein levels at the 50 ppm concentration (Sokal *et al.*, 1980). Exposure to the higher concentrations resulted in increased liver and kidney weights and dose-dependent increases in incidence of lesions of the liver and testis. Serum enzymes were also elevated in the 500 and 20,000 ppm groups.

Similarly, Winell *et al.* (1976) found decreased body weights and increased serum alkaline phophatase levels in mice exposed continuously to 50 or 500 ppm vinyl chloride, compared to controls. In addition, mean survival time was 46 weeks in the 50 ppm group and 35 weeks in the 500 ppm group.

In a series of experiments, various animals (rats, rabbits, guinea pigs, and dogs) were exposed to 3 different regimens of vinyl chloride (Torkelson *et al.*, 1961). Concentrations were 0, 50, 100, 200, and 500 ppm, 7 hours/day, 5 days/week for periods of 4.5 months, 180 days, or 204 days. No effects were observed in animals exposed to 50 ppm vinyl chloride, compared to control animals. Exposures of 100 ppm resulted in increased liver weights in rats after 138-144 exposures in 204 days. Rabbits exposed to 200 ppm showed hepatic lesions, including necrosis and cellular infiltration. Exposure to 500 ppm resulted in liver and renal histological lesions in all animals.

Lee and associates (1977) exposed mice and rats to VC at levels of 0, 50, 250, or 1000 ppm for 6 hours/day, 5 days/week for up to 12 months. By 9 months, all mice exposed to 1000 ppm VC had died. All female mice exposed to 250 ppm died. Death was preceded by rough coat, anorexia, and rapid weight loss. Liver DNA synthesis was increased in the 50 ppm group. In the rats, 8 males and 13 females died in the 1000 ppm exposure groups, while 4 males and 10 females died in the 250 ppm groups. Hepatic and renal damage were observed in the animals that died early in the exposure periods.

Lester *et al.* (1963) found increased liver weights and decreased spleen weights in rats (12-15 per group) exposed 8 hours/day, 5 days/week for 89-92 days to 2% (2000 ppm) VC vapor. Peripheral total leukocyte and neutrophil counts were also depressed.

A continuous 62-day exposure of mice (4-5 per group) to 0 or 30-40 ppm VC resulted in increased basophilic stippled erythrocytes in the peripheral blood, with peak levels occurring after 24 days of exposure (Kudo *et al.*, 1990). Similar findings occurred following intermittent, 4 hours/day, 4-5 days/week exposures.

Pregnant mice (30-40 per group), rats (20-35 per group), and rabbits (15-25 per group) were exposed to vinyl chloride 7 hours/day on gestational days 6-15 (mice and rats) or 6-18 (rabbits) (John *et al.*, 1981). Concentrations of VC were 0, 50, or 500 ppm in the mice, and 0, 500, or 2500 in the rats and rabbits. Maternal mortality was observed in the mice exposed to 500 ppm (5 deaths out of 29 animals). Additionally, body weight, food consumption, and absolute liver weights were decreased compared to controls. Litter size and fetal body weights were smaller while the number of resorbed implantations and skeletal anomalies were greater in mice treated with 500 ppm VC. One rabbit and one rat died in the 2500 ppm groups. Liver weights were increased over controls in rats exposed to 2500 ppm. At the 500 ppm concentration, a decrease in number of corpora lutea/dam was observed, compared with controls. Rabbits exhibited decreased litter size at 500 ppm, as well as increased incidence of unossified sternebrae.

Ungvary *et al.* (1978) found that exposure of rats (13-28 litters) to 1500 ppm vinyl chloride on days 1-9 of gestation resulted in increased maternal liver weights and an increase in the number of resorbed fetuses as a percentage of implants, compared to controls.

VI. Derivation of Chronic Reference Exposure Level (REL)

Study	Ho *et al.* (1991)
Study population	271 workers
Exposure method	Occupational
Critical effects	Abnormal serum biochemistry, indicating impaired liver function; abnormal liver scans, and abnormal liver biopsies
LOAEL	1.5 ppm (0.6 - 10 ppm) (mean and range)
NOAEL	Not observed
Exposure continuity	8 hours/day (10 m^3/day occupational inhalation rate), 5 days/week (assumed)
Exposure duration	5.1 years (range of 1 to 13 years)
Average occupational exposure	0.54 ppm for LOAEL group (1.5 x 10/20 x 5/7)
Human equivalent concentration	0.54 ppm for LOAEL group
LOAEL uncertainty factor	10
Subchronic uncertainty factor	3
Interspecies uncertainty factor	1
Intraspecies uncertainty factor	10
Cumulative uncertainty factor	300
Reference exposure level	0.002 ppm (2 ppb; 0.005 mg/m^3; 5 μg/m^3)

Twelve of 271 workers in a vinyl chloride polymerization plant exhibited abnormal liver function tests (glutamic pyruvic transaminase and gamma glutamyl transpeptidase) in addition to abnormal non-cancerous liver biopsies. Liver scans showed hepato/splenomegaly in 10 of the 12 workers. The 12 workers were all under age 35 at the time of diagnosis. The geometric mean VC concentrations were 5.9 ppm in 1976 and 1.5 ppm in 1983. Ten out of the 12 workers improved their liver function test results within 6 months to 2 years after removal from further VC exposure. Of the 8 workers who returned to working under exposure to VC, all liver function tests became abnormal again within 3 months to a year. Since the first appearance of abnormal liver function occurrred in workers after only 1 year of exposure, and since relapse of liver dysfunction occurred in all 8 returning workers after only 3 months re-exposure to VC, the exposure required for the adverse effect was considered subchronic.

The uncertainty factor for sensitive subgroups was retained since a sensitive individual would likely have some form of compromised liver function independent of their prior exposure to VC (e.g. people with hepatitis, cirrhosis, etc.). Although compared with the remaining workers without hepatotoxicity, these workers are a sensitive group, there were no predisposing liver conditions in these workers prior to VC exposure. The presence of such a predisposing condition would likely make the hepatotoxic response to chronic VC exposure much more severe.

In a related study, a trend for increasing frequency of abnormal liver function tests with increasing VC exposure was found in workers (Liss *et al.*, 1985). Specifically, impaired indocyanine green clearance and elevated serum bile levels were associated with increasing VC exposure. The statistical tests performed using exposure categories did not allow for determination of a NOAEL or LOAEL from the study.

The strengths of the inhalation REL include the use of human exposure data from workers exposed over a period of years. Major areas of uncertainty are the uncertainty in estimating exposure, the potential variability in exposure concentration, and the lack of observation of a NOAEL.

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CHRONIC TOXICITY SUMMARY

XYLENES

Xylol or commercial xylenes (mixture of 60-70% m- and remaining percentage is mix of o- and p- xylenes), technical grade xylenes or mixed xylenes (20% o-xylene, 40% m-xylene, 20% p-xylene, 20% ethyl benzene, and traces of toluene and C9 aromatics), o-xylene (1,2-dimethylbenzene or 2-xylene), m-xylene (1,3-dimethylbenzene or 3-xylene), p-xylene (1,4-dimethylbenzene or 4-xylene), also noted as methyltoluene, benzene-dimethyl, dimethylbenzene.

CAS Registry Numbers.: 1330-20-7 (technical mixture of o-, p-, and m-xylene); 95-47-6 (o-xylene); 108-38-3 (m-xylene); 106-42-3 (p-xylene)

I. Chronic Toxicity Summary

Inhalation reference exposure level	**200 µg/m^3** (for technical or mixed xylenes or sum of individual isomers of xylene)
Critical effect(s)	CNS effects in humans; irritation of the eyes, nose, and throat
Hazard index target(s)	Nervous system; respiratory system

II. Physical and Chemical Properties (ATSDR, 1995; HSDB, 1995)

Description	Colorless liquid
Molecular formula	C_8H_{10}
Molecular weight	106.16 g/mol
Specific gravity	0.864 @ 20°C/4°C (technical mixture); 0.881 (o-); 0.860 (m-); 0.861 (p-)
Boiling point	137-140°C @ 760 mm Hg (technical mixture); 144.4°C (o-); 139.1°C (m-); 138.4°C (p-)
Vapor pressure	6.6 mm Hg (o-); 8.39 mm Hg (m-); 8.87 mm Hg (p-) all @ 25°C.
Solubility	Practically insoluble in water; miscible with absolute alcohol, ether and many other organic solvents
Conversion factor	1 ppb = 4.34 µg/m^3

III. Major Uses or Sources

Mixtures of o-, p-, and m- isomers are extensively used in the chemical industry as solvents for products including paints, inks, dyes, adhesives, pharmaceuticals, and detergents (HSDB, 1995). In the petroleum industry xylenes are used as antiknock agents in gasoline, and as an

intermediate in synthetic reactions. Of the three isomers, p-xylene is produced in the highest quantities in the U.S. for use in the synthesis of phthalic, isophthalic, and phthalicterephthalic acid used in manufacture of plastics and polymer fibers including mylar and dacron.

IV. Effects of Human Exposure

Information on the toxicity of xylenes to humans is almost exclusively limited to case reports of acute exposures and studies of occupational exposures in which persons often inhaled a mixture of hydrocarbon solvents 8 hours per day, 5-6 days per week; these studies often have incomplete information on the airborne concentrations of xylene and other hydrocarbons. One study examining chronic effects in humans from inhalation of predominantly mixed xylenes was identified (Uchida *et al.*, 1993) and one study examining subchronic effects of p-xylene exposure was identified (Hake *et al.*, 1981). No studies examining the chronic effects of oral or dermal xylene exposure in humans were identified.

Pharmacokinetic studies have documented the absorption of xylene in humans through inhalation, oral, and dermal routes of exposure. Approximately 60% of inspired xylene is retained systemically (Sedivec and Flek, 1979). The majority of ingested xylene (~90%) is absorbed into the systemic circulation (ATSDR, 1995). Xylene is also absorbed dermally; the rate of absorption of xylene vapor is estimated as 0.1-0.2% of that by inhalation (Riihimaki and Pfaffli 1978). Measurement of the rate of absorption through direct contact with the skin produced variable results ranging from 2 $\mu g/cm^2$/min (Engstrom *et al.*, 1977) to 75-160 $\mu g/cm^2$/min (Dutkiewicz and Tyras, 1968).

Xylene exposure has been associated with effects in a number of organ systems including the lungs, skin and eyes; neurological system; heart and gastrointestinal system; kidney; and possibly the reproductive system.

Pulmonary effects have been documented in occupational exposures to undetermined concentrations of mixed xylenes (and other solvents) and include labored breathing and impaired pulmonary function (Hipolito 1980; Roberts *et al.*, 1988). High levels of xylene exposure for short periods are associated with irritation of the skin, eyes, nose and throat (ATSDR, 1995). Chronic exposure to xylenes has been associated with eye and nasal irritation (Uchida *et al.*, 1993).

The central nervous system is affected by both short term and long term exposure to high concentrations of xylene with: 100-200 ppm associated with nausea and headache; 200-500 ppm with dizziness, irritability, weakness, vomiting, and slowed reaction time; 800-10,000 ppm with lack of muscle coordination, giddiness, confusion, ringing in ears, and changes in sense of balance; and >10,000 ppm with loss of consciousness (HESIS, 1986). Other neurological effects documented include impaired short term memory, impaired reaction time, performance decrements in numerical ability, and impaired equilibrium (dizziness) and balance (Carpenter *et al.*, 1975; Dudek *et al.*, 1990; Gamberale *et al.*, 1978; Riihimaki and Savdainen, 1980;

Savolainen and Linnavuo, 1979; Savolainen and Riihimaki 1981; Savolainen *et al.*, 1979; 1984; 1985).

Chronic exposure to xylenes (with other hydrocarbons) have been associated with cardiovascular and gastrointestinal effects. Heart palpitations, chest pain, and abnormal electrocardiogram were noted (Hipolito, 1980; Kilburn *et al.*, 1985) as were effects on the gastrointestinal system producing nausea, vomiting and gastric discomfort in exposed workers (Goldie, 1960; Hipolito, 1980; Uchida *et al.*, 1993; Klaucke *et al.*, 1982; Nersesian *et al.*, 1985).

Results of studies of renal effects of xylene are mixed and come from case reports and occupational studies where multiple chemical exposures are common. The effects from subchronic exposure documented by Hake *et al.* (1981) and from chronic exposure documented by Uchida *et al.* (1993) did not include renal effects. However, Morley *et al.* (1970) found increased BUN and decreased creatinine clearance; Martinez *et al.* (1989) found distal renal tubular acidemia; Franchini *et al.* (1983) found increased levels of urinary b-glucuronidase; and Askergren (1981, 1982) found increased urinary excretion of albumin, erythrocytes, and leukocytes.

Reproductive effects were documented by Taskinen *et al.* (1994) who found increased incidence of spontaneous abortions in 37 pathology and histology workers exposed to xylene and formaldehyde in the work place. The multiple chemical exposures and the small number of subjects in this study limit the conclusions that can be drawn as to reproductive effects of xylene in humans.

No hematological effects have been identified in studies where exposure was to xylene only. Previous studies identifying hematological effects included known or suspected exposure to benzene (ATSDR, 1995; ECETOC, 1986). One series of case reports identified lowered white cell counts in two women with chronic occupational exposure to xylene (Hipolito, 1980; Moszczynsky and Lisiewicz, 1983; 1984), although they may also have had multiple chemical exposures.

The Uchida *et al.* (1993) study included a relatively large number of workers studied, exposure for an average of 7 years to xylenes predominately and a comprehensive set of medical examinations to document potential effects. A survey of 994 Chinese workers involved in the production of rubber boots, plastic coated wire and printing processes employing xylene solvents was carried out. The survey consisted of fitting individual workers with diffusive samplers for an 8 hour shift. At the end of the 8 hour shift the samplers were recovered for analysis of solvent exposure, and urine samples were collected for analysis of xylene metabolites. The following day workers answered a questionnaire concerning subjective symptoms, and blood and urine were collected for analysis. Out of this group of xylene-exposed workers, 175 individuals (107 men and 68 women) was selected for further study and analysis based on completion of their health examinations and results from diffusive samplers showing that xylene constituted 70% or more of that individual's exposure to solvents in the workplace. A control population consisting of 241 (116 men and 125 women) unexposed workers from the same factories or other factories in the same region, of similar age distribution, of similar time in this occupation (average of 7

years), and having a similar incidence of alcohol consumption and cigarette usage were selected. The xylene-exposed and unexposed groups were given health examinations evaluating hematology (red, white, and platelet cell counts, and hemoglobin concentration); serum biochemistry (albumin concentration, total bilirubin concentration, aspartate aminotransferase, alanine aminotransferase, gamma-glutamyl transferase, alkaline phosphatase, leucine aminopeptidase, lactate dehydrogenase, amylase, blood urea nitrogen, creatinine); and subjective symptoms (survey of symptoms occurring during work and in the previous three months).

Results of analysis of the diffusive samplers showed that workers were exposed to a geometric mean of 14.2 ± 2.6 ppm xylene (arithmetic mean of 21.3 ± 21.6 ppm). This was broken down into geometric means of 1.2 ppm o-xylene, 7.3 ppm m-xylene, 3.8 ppm p-xylene, 3.4 ppm ethyl benzene, and 1.2 ppm toluene. n-Hexane was rarely present and no benzene was detected. Analysis of data from the health examinations found no statistically significant difference ($p<0.10$) between hematology and serum biochemistry values for xylene-exposed and unexposed populations. The frequency of an elevated ratio of aspartate aminotransferase to alanine transferase and elevated ratio of alkaline phosphatase to leucine aminopeptidase was significantly ($p<0.01$) higher in exposed men than in the control population of men. Results of the survey of subjective symptoms found differences in symptoms occurring during work and over the previous three months apparently related to effects on the central nervous system and to local effects on the eyes, nose and throat. The frequency of five symptoms experienced during work were significantly ($p<0.01$) elevated in either xylene-exposed men or women including: dimmed vision, unusual taste, dizziness, heavy feeling in the head, and headache. The frequency of four symptoms experienced during work were significantly ($p<0.01$) elevated in both men and women including irritation in the eyes, nasal irritation, sore throat, and floating sensation. Ten subjective symptoms occurring in the previous three months were significantly ($p<0.01$) elevated in exposed men and women including nausea, nightmare, anxiety, forgetfulness, inability to concentrate, fainting after suddenly standing up, poor appetite, reduced grasping power, reduced muscle power in the extremities, and rough skin. Dose dependency appeared to exist for 3 subjective symptoms noted during work: irritation in the eyes, sore throat, floating sensation, and one symptom occurring in the last three months, poor appetite.

V. Effects of Animal Exposure

A limited number of chronic toxicity studies are available for xylene including two inhalation studies with o-xylene (Tatrai *et al.*, 1981; Jenkins *et al.*, 1970) and one oral chronic study with mixed xylenes (NTP, 1986). No chronic dermal studies could be identified. A spectrum of adverse effects has been documented in shorter term studies which potentially could occur with chronic exposure. These studies are presented here along with a brief description of the three chronic studies identified. Xylene affects a number of organ systems including the pulmonary system, the cardiovascular system, the gastrointestinal system, the hepatic system, the renal system, the dermis and the eye and has numerous neurological effects and developmental effects.

Animal data is consistent with human data in documenting respiratory effects from xylene exposure. Acute and subacute exposures in mice, rats, and guinea pigs have been associated with

decreased metabolic capacity of the lungs; decreased respiratory rate; labored breathing; irritation of the respiratory tract; pulmonary edema; and pulmonary inflammation (Carpenter *et al.*, 1975; De Ceaurriz *et al.*, 1981; Elovaara *et al.*, 1987; 1989; Furnas and Hine, 1958; Korsak *et al.*, 1988; 1990; Patel *et al.*, 1978; Silverman and Schatz, 1991; Toftgard and Nilsen, 1982).

Limited evidence is available in animal studies for cardiovascular effects resulting from xylene exposure. Morvai *et al.* (1976; 1987) conducted two studies. The first study observed rats following acute and intermediate duration inhalation exposure to very high (unspecified) levels of xylene and recorded ventricular repolarization disturbances, atrial fibrillation, arrhythmias, occasional cardiac arrest and changes in electrocardiogram (Morvai *et al.*, 1976). In a subsequent study morphological changes in coronary microvessels were seen in rats exposed to 230 ppm xylene (isomer composition unspecified) (Morvai *et al.*, 1987). However the chronic toxicity studies conducted by the National Toxicology Program (NTP, 1986) and by Jenkins *et al.* (1970), as well as other shorter term studies (Carpenter *et al.*, 1975; Wolfe, 1988), have not identified histopathological lesions of the heart.

Studies identifying adverse gastrointestinal effects, hematological effects, or musculoskeletal effects in animals were not identified. Studies reporting no hematological effects include Carpenter *et al.* (1975) (rats exposed to 810 ppm of mixed xylenes for 10 weeks, 5 days/week, 6 hours/day and dogs exposed for 13 weeks to 810 ppm mixed xylenes, 5 days/week, 6 hours/day) and Jenkins *et al.* (1970) (rats, guinea pigs and dogs exposed for 6 weeks to 780 ppm o-xylene, 5 days/week, 8 hours per day). Carpenter *et al.* (1975) and the NTP (1986) reported no effects on the musculoskeletal system.

Hepatic effects have been documented after acute exposure to high concentrations of xylene (2,000 ppm) or subacute exposure to lower concentrations (345-800 ppm) of mixed xylene or individual isomers. These effects include increased cytochrome P-450 and b5 content, increased hepatic weight, increased liver to body weight ratios, decreased hepatic glycogen, proliferation of endoplasmic reticulum, changes in distribution of hepatocellular nuclei, and liver degeneration (Bowers *et al.*, 1982; Condie *et al.*, 1988; Elovaara, 1982; Elovaara *et al.*, 1980; Muralidhara and Krishnakumari 1980; Patel *et al.*, 1979; Pyykko 1980; Tatrai and Ungvary, 1980; Tatrai *et al.*, 1981; Toftgard and Nilsen, 1981; 1982; Toftgard *et al.*, 1981; Ungvary *et al.*, 1980).

Renal effects have been identified in studies with rats, guinea pigs, dogs, and monkeys exposed to 50-2,000 ppm of xylenes. These effects include increased cytochrome P-450 content and increased kidney to body weight ratios (Condie *et al.*, 1988; Elovaara 1982; Toftgard and Nilsen, 1982). Condie *et al.* (1988) also noted tubular dilation, atrophy, and increased hyaline droplets in the kidney of Sprague-Dawley rats administered 150 mg/kg/day orally of mixed xylenes. This response is consistent with early nephropathy.

Xylene has been found to effect the dermis and eyes of animals. Hine and Zuidema (1970) found skin erythema and edema, epidermal thickening, and eschar formation in response to xylene exposure. Direct instillation of xylenes into the eyes of rabbits produces eye irritation (Hine and Zuidema, 1970; Smyth *et al.*, 1962)

Numerous neurological effects have been documented in response to acute and subchronic xylene exposures ranging between 160 to 2,000 ppm. This is consistent with effects on neurofunction documented in humans. These effects include narcosis, prostration, incoordination, tremors, muscular spasms, labored respiration, behavioral changes, hyperactivity, elevated auditory thresholds, hearing loss, and changes in brain biochemistry (Andersson *et al.*, 1981; Carpenter *et al.*, 1975; De Ceaurriz *et al.*, 1983; Furnas and Hine, 1958; Ghosh *et al.*, 1987; Kyrklund *et al.*, 1987; Molnar *et al.*, 1986; NTP, 1986; Pryor *et al.*, 1987; Rank 1985; Rosengren *et al.*, 1986; Savolainen and Seppalainen, 1979; Savolainen *et al.*, 1978; 1979a; Wimolwattanapun *et al.*, 1987).

Developmental effects have been documented in pregnant animals exposed to xylenes. ATSDR (1995) concluded that the body of information available for developmental effects are consistent with the hypothesis that xylene is fetotoxic and many of the fetotoxic responses are secondary to maternal toxicity. Exposure of pregnant rats and mice to 500 ppm of mixed xylenes or 350-700 ppm of xylene isomers increased fetal death, decreased fetal weight, delayed skeletal development, produced skeletal abnormalities, produced enzymatic changes in fetal organs, and produced maternal toxicity (Hudak and Unvary, 1978; Marks *et al.*, 1982; Mirkova *et al.*, 1983; Ungvary *et al.*, 1980; 1980a; 1981). Marks *et al.* (1982) also noted that 2,060 mg/kg/day of mixed xylene administered orally is associated with cleft palate and decreased fetal weight. Potential neurological/muscular changes, measured as performance on a rotorod, were noted in 2-day-old rat pups exposed to 200 ppm mixed xylenes on days 4-20 of gestation (Hass and Jakobsen, 1993). These results are consistent with Mirkova *et al.* (1979) who documented biochemical changes in fetal and maternal brain tissues in response to dermal exposure to xylenes.

Of the three chronic studies available (Tatrai *et al.*, 1981; Jenkins *et al.*, 1970; NTP 1986) none comprehensively examined systemic effects. The study by Tatrai *et al.* (1981) exposed rats for one year, 7 days/week, 8 hours per day to 1096 ppm o-xylene. This exposure was a LOAEL for body weight gain in males and a NOAEL for hepatic effects in male rats. Jenkins *et al.* (1970) exposed rats, guinea pigs, squirrel monkeys, and beagle dogs for 90-127 days continuously to 78 ppm of o-xylene. The study examined body weight gain; hematological parameters including white cell counts, red blood cell counts, and hematocrit; serum biochemistry including bromosulfalein retention, blood urea nitrogen, alanine aminotransferase, aspartate aminotransferase, alkaline phosphatase, and creatinine and liver function including alkaline phosphatase, tyrosine aminotransferase and total lipids. No effects were observed in any of the parameters examined in this study. This study found a NOAEL for all effects examined of 78 ppm o-xylene. The NTP (1986) study administered 0, 250, or 500 mg/kg/day doses of mixed xylene in corn oil by gavage 5 days/week for 103 weeks to groups of F344/N rats of both sexes, 50 animals per group. B6C3F1 mice were treated in a similar manner but given 0, 500 or 1000 mg/kg/day of mixed xylenes in corn oil by gavage. A complete histopathological examination of all tissues were made as well as determination of body weight gain. Based on histopathology of all organ systems, a NOAEL of 500 ppm was observed for rats and a NOAEL of 1000 ppm was observed for mice.

VI. Derivation of Chronic Reference Exposure Level (REL)

Study	Uchida *et al.* (1993)
Study population	175 xylene-exposed factory workers and control population of 241 factory workers
Exposure method	Inhalation
Critical Effects	Dose related increase in the prevalence of eye irritation, sore throat, floating sensation, and poor appetite.
LOAEL	14.2 ppm (geometric mean of exposure concentrations)
NOAEL	Not applicable
Exposure continuity	Occupational exposure for an average of 7 years
Average exposure concentration	5.1 ppm for LOAEL group (14.2 x 10/20 x 5/7)
Human equivalent concentration	5.1 ppm for LOAEL group
LOAEL uncertainty factor	10
Subchronic uncertainty factor	1
Interspecies uncertainty factor	1
Intraspecies uncertainty factor	10
Cumulative uncertainty factor	100
Inhalation reference exposure level	0.05 ppm (50 ppb; 0.2 mg/m^3; 200 µg/m^3) for mixed xylenes or for total of individual isomers

A number of issues are important in considering the uncertainty associated with this REL. ATSDR (1995) concludes that animal and human toxicity data suggest that mixed xylenes and the different xylene isomers produce similar effects, although different isomers are not equal in potency for producing a given effect. Therefore exposure of workers to a mix of xylenes in the Uchida *et al.* (1993) study would be expected to generate a similar spectrum of responses as exposure to single isomers, however the intensity of particular effects could be different. The use of a neurological endpoint for derivation of a REL is supported by the large number of inhalation and oral studies associating neurological effects with xylene exposure. ATSDR (1995) indicates that neurological effects are a sensitive endpoint. The observation that floating sensation is apparently related to dose further supports the concept that this subjective symptom related to neurological effects was due to xylene exposure. The use of a factor of 10 for using a LOAEL as the basis for the REL should serve to protect populations from neurological effects as should the use of a factor of 10 for sensitive individuals within the population. Another issue is the use of diffusive samplers in the Uchida *et al.* (1993) study. These samplers provide a time weighted average concentration of hydrocarbon and cannot indicate the maximum concentrations a worker is exposed to. It is unknown whether peak concentrations alter the response to xylenes in humans.

The strengths of the inhalation REL include the use of human exposure data from workers exposed over a period of years. Major areas of uncertainty are the uncertainty in estimating

exposure and the potential variability in exposure concentration, and the lack of observation of a NOAEL.

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CHRONIC TOXICITY SUMMARY

ZINC AND ZINC COMPOUNDS

Molecular Formula	*Synonyms*	*Molecular Weight*	*CAS Reg. No.*
Zn	metallic zinc; merrillite; blue powder	65.4	7440-66-6
ZnO	zinc oxide; zincite; zinc white	81.4	1314-13-2
$ZnCl_2$	zinc chloride; butter of zinc; tinning flux	136.3	7646-85-7

I. Chronic Toxicity Summary

Inhalation reference exposure level	**0.9 µg Zn/m^3**
Critical effect(s)	Development of occupational asthma in an exposed human
Hazard index target(s)	Respiratory system; immune system

II. Chemical Property Summary (HSDB, 1995)

Molecular formula	See above
Molecular weight	See above
Description	Zn: blue-white lustrous metal $ZnCl_2$: white crystals ZnO: white/yellowish/grayish powder
Vapor pressure	Zn: 1 mm Hg @ 487°C $ZnCl_2$: 1 mm Hg @ 428°C ZnO: not applicable
Solubility	Zn: sol. in acids, alkalis, and acetic acid $ZnCl_2$: sol. in water, alcohol, glycerol, acetone, ether ZnO: sol. in dil. acids, ammonia salts; insol. in alcohol
Conversion factor	Not applicable for particulates

III. Major Uses and Sources

Zinc metal is used as a component of numerous metal alloys (in brass and bronze and with gold and magnesium), and is used as a corrosion inhibitor on items such as eating utensils, automotive equipment, and castings (HSDB, 1995). It is also used as a galvanizing agent.

The primary uses of zinc chloride ($ZnCl_2$) are in manufacturing, particularly certain dyes, glues, textiles, artificial silk, and parchment paper (HSDB, 1995). It is a component of some asphalts, dental cements, deodorants, dentifrices, and embalming fluids and has uses as a preservative for wood, soldering flux and a corrosion inhibitor. $ZnCl_2$ is also a major component of some smoke bombs used in crowd control. Other uses include the galvanization of iron and the refining of petroleum (ACGIH, 1992). Zinc is also used as a human dietary supplement.

The primary uses of zinc oxide (ZnO) are in paint pigments, cosmetics, and cements; in the manufacture of floor coverings, fabrics, lubricants, plastics, rayon, glass, enamels, glue, matches, semiconductors; and as an anticaking agent. Fumes of ZnO may be generated from the heating of zinc metal or zinc alloys (as in welding or soldering). ZnO and $ZnCl_2$ have medical uses as astringents and veterinary antiseptics (HSDB, 1995; ACGIH, 1992).

IV. Effects of Human Exposures

Exposure to airborne zinc in the form of dust or fumes is most commonly associated with the condition termed “metal fume fever”, characterized by cough, difficulty in breathing, chills, chest pain, nausea, vomiting, and leukocytosis in the lungs (ACGIH, 1992; Vogelmeier *et al.*, 1987). This condition results from acute exposures to zinc fumes and generally resolves without treatment between exposures; some workers may develop the condition repeatedly during the course of employment. There is no evidence that repeated exposure results in a cumulative toxic effect of this type. Some studies with human subjects have attempted to address the exposure level necessary to elicit this response. Eight hour exposure to 14 mg Zn/m^3 and 20 minute exposure to 45 mg Zn/m^3 failed to produce this effect in humans (Drinker *et al.*, 1927; Hammond, 1944 (see below)).

A hazard evaluation was made for quarry workers (number not stated) in the crushed stone industry showing symptoms of “zinc chills” plus chest pains and difficulty in breathing 2 to 12 hours after exposure, with subsequent pneumonia developing occasionally (Hammond, 1944). Exposure resulted when molten zinc metal was used to fill in the lining areas around the jaw crushers. The primary contaminants of the zinc metal used were lead, tin and copper which represented < 4% impurity. Three exposure levels measured at the time of pouring the lining showed an average of 12 mg $dust/m^3$ with 10 mg Zn/m^3. The author noted no symptoms in himself after unprotected exposure to these levels during sampling. Workers involved in the cutting/removing of the lining with oxygen-acetylene torches were exposed to average concentrations of 465 mg Zn/m^3, 12.4 mg Mn/m^3, and 1.6 mg Pb/m^3, with an 8-hour time-weighted average zinc exposure of 85 mg/m^3.

A case report was published showing the development of occupational asthma in a worker exposed to zinc from galvanization processes over a 2 year period (Malo *et al.*, 1993). Environmental monitoring resulted in estimated exposure levels of 0.26-0.29 mg Zn/m^3 and 0.03-0.13 mg Fe/m^3. Over the course of employment, the worker experienced increasing shortness of breath, chest tightness, wheezing, sneezing, and burning of the eyes. Symptoms disappeared when he left work for seven months, but reappeared when he returned to work. The

patient was examined 3 months after leaving work the second time. Methacholine challenge indicated mild airway hyperresponsiveness. Serial spirometry, measured during the course of a 7 hr workday, showed a 24% decline in FEV_1 compared with no change measured during the course of a one day hospital stay. Inhalation challenge with a 10 mg $ZnSO_4$/ml solution over 6 minutes also resulted in an immediate reaction, reducing FEV_1 a maximum of 23%. Dermal application of 1 and 10 mg/ml of $ZnSO_4$, but not copper, chromium, or cobalt, resulted in an immediate skin reaction. The response did not appear to be IgE-mediated, however. Acute and late phase reactions have also been characterized in a welder exposed to zinc fumes, with the appearance of hives and angioedema developing in an "immediate and delayed fashion" (Farrell, 1987).

Fourteen welders acutely exposed to 77-153 mg Zn/m^3 as ZnO were reported to have an increased proportion of activated T-cells and increased numbers of immune cells including neutrophils, macrophages, and lymphocytes 20 hours after exposure. (Blanc *et al.*, 1991). Smelter workers exposed to zinc fumes (level unspecified) also showed increased lymphocytes (Ameille *et al.*, 1992).

Smoke containing zinc chloride is acutely toxic to the respiratory system, with many case reports of injury associated with exposure (Matarese and Matthews, 1986; Hjortsø *et al.*, 1988; Schenker *et al.*, 1981; Milliken *et al.*, 1963; Evans, 1945). Adverse effects have included acute respiratory distress, interstitial fibrosis, irritation, cough, and nausea. Long-term respiratory effects in humans from exposure to $ZnCl_2$, however, have not been documented.

V. Effects of Animal Exposures

Mortality was reported to be 50% in mice (vs. 20% in controls) and 22% in guinea pigs (vs. 8% in controls) exposed to ~120 mg Zn/m^3 for 1 hr/day for 3-20 weeks in the form of $ZnCl_2$ smoke (Marrs *et al.*, 1988). Other effects included focal alveolitis, emphysema, and fibrosis after 3 weeks of exposure. Increased lung macrophages were observed in rats and mice 13 months following 20 weeks of exposure. The smoke also contained ZnO, hexachloroethane, and other compounds.

Pulmonary effects of zinc oxide were examined in guinea pigs exposed to 3.7 or 4.3 mg Zn/m^3 as ZnO for 3 hrs/day for 6 days (Lam *et al.*, 1985). The exposure resulted in changes in pulmonary function (decreased compliance, increased flow resistance, decreased diffusing capacity) and morphological changes (inflammation, leukocytosis, interstitial thickening, increased lung weight). Morphological changes were still present 72 hours after the last exposure. Inhalation exposure of guinea pigs to 5.6 mg Zn/m^3 , but not 2.2 mg Zn/m^3, for 3 hrs/day for 5 days resulted in decreased total lung capacity, vital capacity, and carbon monoxide diffusing capacity (Lam *et al.*, 1988).

Male guinea pigs (3/group plus 6 control animals) were exposed for 3 hrs/day for 1, 2, or 3 days in nose-only exposure chambers to 0, 2.3, 5.9 or 12.1 mg ZnO/m^3 in the form of ultrafine particles (< 1 μm), with the different groups exposed on consecutive weeks (Conner *et al.*, 1988).

Lungs and pulmonary lavage fluid were examined by light microscopy. Animals in the two highest dose groups showed evidence of centriacinar inflammation and increased protein and neutrophils in the lavage fluid. Lavage fluid also exhibited increased acid phosphatase and lactate dehydrogenase activity. Male guinea pigs (N=23) similarly exposed for 1 hr to ~1 mg ZnO/m^3 (fume) showed a decrease in lung compliance both 1 and 2 hrs after exposure (Amdur *et al.*, 1982). Other pulmonary function parameters were unchanged.

Pulmonary macrophage function, as measured by uptake of colloidal gold, was measured in male hamsters (6-12/group) exposed for 4 hours to 0, 0.8, 3.1, 6.5, and 20.3 mg SO_4^{2-}/m^3 generated by the aerosolization of $ZnSO_4$ (median aerodynamic diameter = 0.59 μm) and 0, 2.5, 5.4, 10.0, 15.0, and 39.6 mg SO_4^{2-}/m^3 generated by the aerosolization of zinc ammonium sulfate [$Zn(NH_4SO_4)_2$] (Skornik and Brain, 1983). Significant reductions in uptake were observed in animals exposed to $ZnSO_4$ one hour after exposure in all dose groups ≥ 3.1 mg/m^3 and 24 hours after exposure in the 6.5 and 20.3 mg/m^3 dose groups. Reductions in uptake were observed in animals exposed to $Zn(NH_4SO_4)_2$ in all dose groups ≥10 mg/m^3 one and 24 hours after exposure.

VI. Derivation of Chronic Reference Exposure Level (REL)

Derivation of Chronic Inhalation Reference Exposure Level (REL)

Study	Malo *et al.*, 1993
Study population	Human (case report)
Exposure method	Occupational inhalation exposure
Critical effects	Decreased lung function; hypersensitivity
LOAEL	0.26 Zn/m^3
NOAEL	Not observed
Exposure continuity	7 hours/day (assumed 10 m^3/day occupational inhalation exposure), 5 days/week
Exposure duration	2 years
Average occupational exposure	0.093 mg Zn/m^3 for LOAEL (0.26 x 10/20 x 5/7)
Human equivalent concentration	0.093 mg Zn/m^3 for LOAEL
LOAEL uncertainty factor	10
Subchronic uncertainty factor	10
Interspecies uncertainty factor	1
Intraspecies uncertainty factor	1
Cumulative uncertainty factor	100
Inhalation reference exposure level	0.0009 mg Zn/m^3 (0.9 μg Zn/m^3)

The primary human health effects which are of concern from chronic inhalation of zinc and its compounds relate to respiratory effects and the development of hypersensitivity in exposed individuals. The study of Malo *et al.* (1993) provides the basis for the development of a chronic REL. This study, although conducted on a single individual, has been deemed useful for several reasons: (1) it was conducted on a human, (2) the subject appears to represent a sensitive

subpopulation (he developed an allergy to zinc), (3) the occupational exposure levels were documented by environmental monitoring, (4) the adverse effect appeared to develop from long-term exposure, (5) subject follow-up clearly indicated a specific sensitivity to zinc, and (6) the study was conducted by a well-established investigative group on hypersensitivity-related health effects. The development of hypersensitivity to zinc fumes had also been previously documented, with the appearance of both an immediate- and a late-phase reaction in a welder (Farrell, 1987).

The most useful of the available animal studies addressing the toxicity of zinc compounds from repeated exposures are those conducted by Lam *et al.* (1985, 1988) and Conner *et al.* (1988) in guinea pigs exposed to ZnO for up to six consecutive days. Respiratory effects including changes in lung function and morphological changes were established showing a dose-response with NOAELs of 2.2 mg Zn/m^3 and 2.3 mg/m^3 for the Lam *et al.* (1988) and Conner *et al.* (1988) studies, respectively. These studies were considered for the development of the chronic REL. Using the 2.2 mg Zn/m^3 NOAEL presented by Lam *et al.* (1988), a chronic REL of 0.3 μg Zn/m^3 is derived following averaging of exposure levels over the course of the experiment (average experimental exposure = 0.275 mg Zn/m^3) and application of uncertainty factors of 10 each for the subchronic duration of the study (5 days), interspecies extrapolation, and potentially sensitive human subpopulations. A study with mice exposed to $ZnCl_2$ smoke over a period of 3-20 weeks is of limited usefulness because of possible contributions of other compounds in the smoke to the toxicity (Marrs *et al.*, 1988). The study exposing hamsters to various metal sulfates including $ZnSO_4$ and $Zn(NH_4SO_4)_2$ and showing decreased alveolar macrophage function is of limited usefulness because, although an effect was observed at 0.8 mg SO_4^{2-}/m^3, the exposure duration was short, leading to uncertainty about potential long-term consequences (Skornik and Brain, 1983).

The strengths of the inhalation REL include the use of human exposure data from workers exposed over a period of years. Major areas of uncertainty are the uncertainty in estimating exposure and the potential variability in exposure concentration, the lack of information about a population of LOAEL, and the lack of observation of a NOAEL.

Derivation of Chronic Oral Reference Exposure Level (REL)

Study	Yadrick *et al.*, 1989
Study population	18 healthy human females
Exposure method	50 mg zinc supplement (plus dietary zinc)
Critical effects	decrease in erythrocyte superoxide dismutase
LOAEL	1 mg/kg-day*
NOAEL	none
Exposure continuity	once per day
Exposure duration	10 weeks
Average experimental exposure	50 mg zinc supplement per day
LOAEL uncertainty factor	3
Subchronic uncertainty factor	(included with LOAEL factor)
Interspecies uncertainty factor	not applicable

Intraspecies factor	1
Cumulative uncertainty factor	3
Oral reference exposure level	0.3 mg/kg-day

*Conversion Factors: The dose conversion factors were based on a 60-kg reference female body weight. Total dose was derived from estimations from the FDA Total Diet Study for 1982-1986, plus reported supplemental dose. For example, for the Yadrick *et al.*, 1989 study, the dose is 1.0 mg/kg-day based on 50 mg zinc supplement plus 9.72 mg/day zinc from the diet (total of 60), divided by the assumed average body weight of the participants (60 kg).

The oral REL is the U.S. EPA's oral Reference Dose (RfD) for zinc. The principal study used was: Yadrick, M.K., M.A. Kenney and E.A. Winterfeldt. 1989. Iron, copper, and zinc status: Response to supplementation with zinc or zinc and iron in adult females. Am. J. Clin. Nutr. 49: 145-150. The oral RfD is based on a clinical study which investigated the effects of oral zinc supplements on copper and iron balance. This study is supported by several other studies which indicate that zinc supplementation can alter copper balance. The effects on copper and iron biochemistry are considered of concern since long-term iron or copper deficiency could result in significant adverse effects. For example, zinc supplementation therapy with megadoses of up to 5 g/day, as well as smaller amounts of 150 mg/day, taken for 1 to 2 years have produced copper deficiency anemia. In addition, several studies have investigated the effects of zinc supplementation on the high-density lipoprotein (HDL) levels of adult males. These have been added as supporting studies because the observed change in HDL values in males may be significant since a sustained decrease in HDL concentrations may be associated with increased risk of coronary artery disease when combined with a parallel increase in low-density lipoprotein (LDL) cholesterol. A 10-week study of zinc supplementation in 18 healthy women given zinc gluconate supplements twice daily (50 mg zinc/day, or 1.0 mg/kg-day, see below) resulted in a decrease of erythrocyte superoxide dismutase (ESOD) activity (Yadrick *et al.*, 1989). ESOD concentrations declined over the 10-week supplementation period and at 10 weeks were significantly different ($p<0.05$) from values during the pretreatment period. By 10 weeks, ESOD activity had declined to 53% of pretreatment levels. Change in enzyme activity is considered a better indicator of altered copper status than a measure of metal concentration in tissue or plasma. This has been documented by studies in rats fed copper-deficient or high-zinc diets, in which copper metalloenzyme activity is greater and precedes changes in plasma or tissue levels of copper. Ceruloplasmin concentrations were not altered. Serum zinc was significantly increased. There was also a significant decline in serum ferritin and hematocrit values at 10 weeks. Such a decrease could pose a significant risk to the iron status of women. No measurements were made of dietary zinc or copper in this study. However, a level of dietary zinc can be estimated at 9.72 mg/day for females (20- to 30-years old) from the results of the FDA Total Diet Study for 1982-1986. The LOAEL of 1.0 mg/kg-day was calculated from the sum of these dietary estimates and the supplemental zinc dose using an assumed body weight of 60 kg for adult females, as shown in the conversion factor section.

An uncertainty factor of 3 was used, based on a minimal LOAEL from a moderate-duration study of the most sensitive humans and consideration of a substance that is an essential dietary nutrient. No modifying factor was used.

The U.S. EPA stated its confidence in the oral RfD as: Study - Medium; Data Base - Medium; and RfD - Medium. The level of confidence in the studies is medium since they are well-conducted clinical studies with many biochemical parameters investigated but only few numbers of humans were tested. The confidence in the overall database is medium since these studies are all of short duration. Medium confidence in the RfD follows.

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